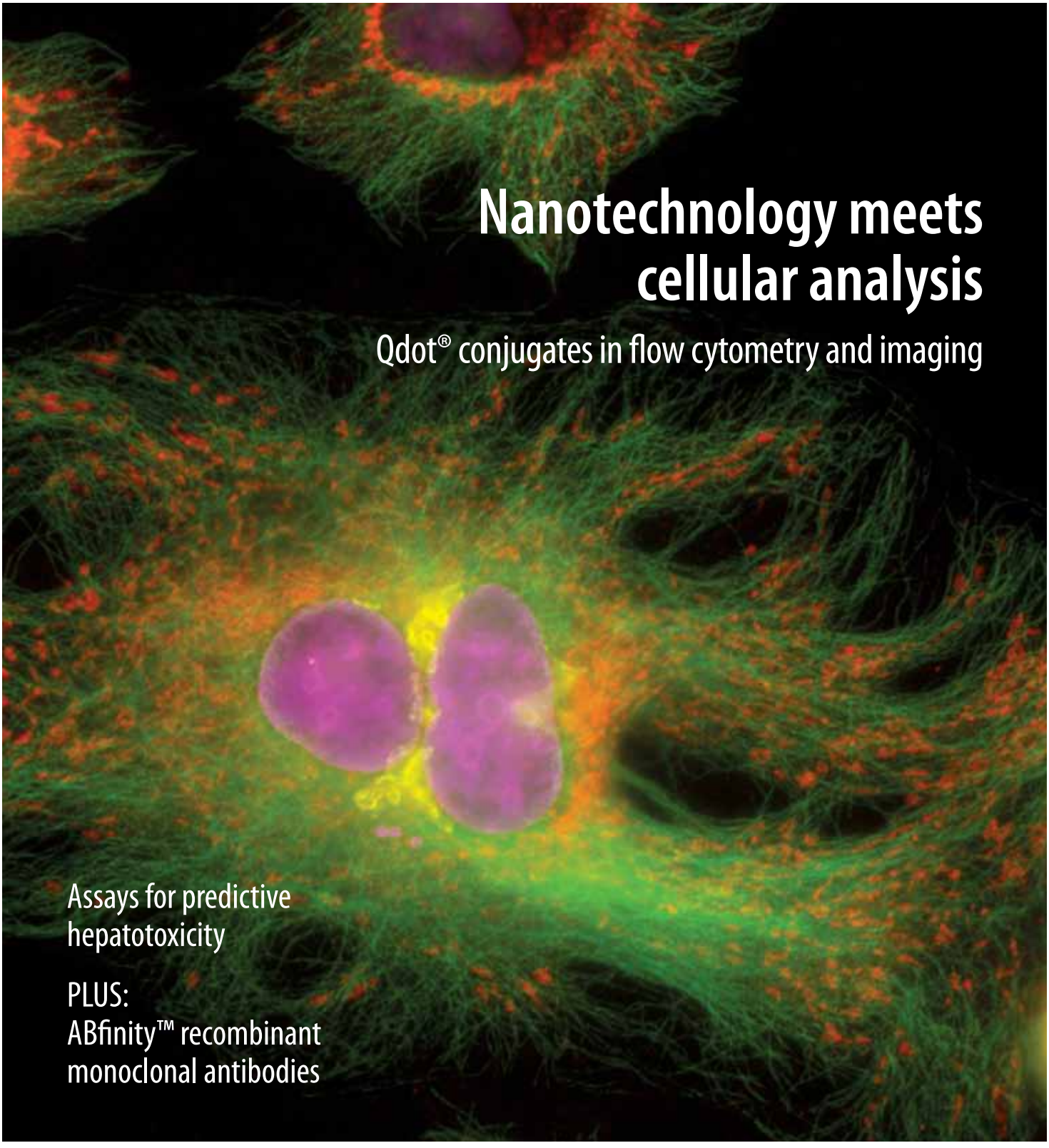
BIOPROBES 61

CELL BIOLOGY BY INVITROGEN

DECEMBER 2009



Nanotechnology meets cellular analysis

Qdot® conjugates in flow cytometry and imaging

Assays for predictive hepatotoxicity

PLUS:
ABfinity™ recombinant monoclonal antibodies

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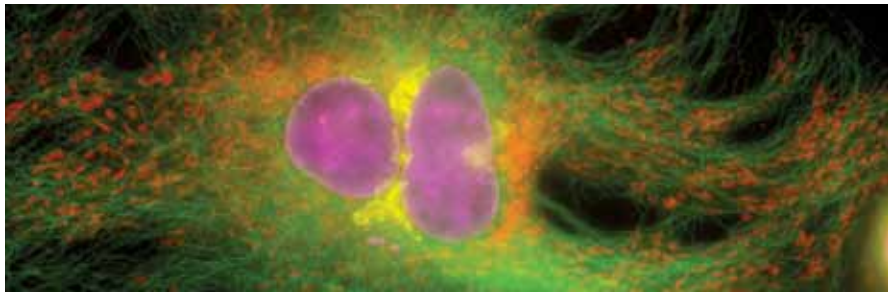

BioProbes 61

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2009 APEX® AWARDS FOR PUBLICATION EXCELLENCE

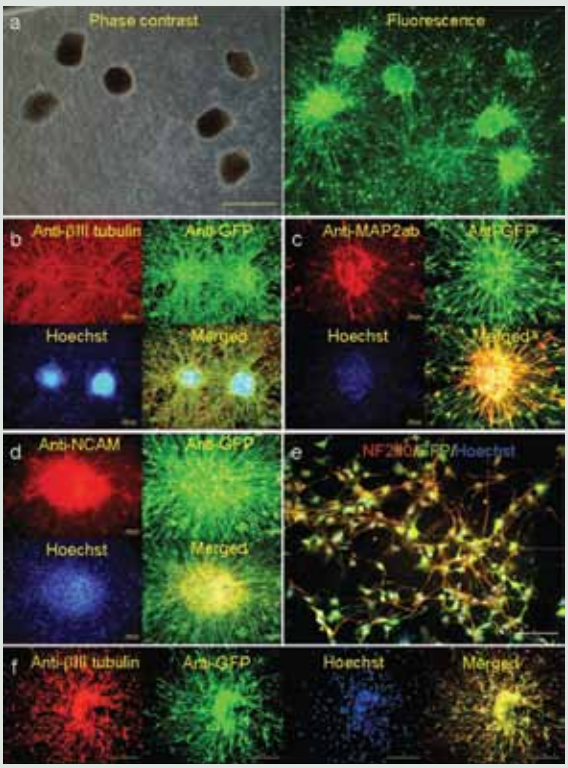
Features

Departments

Baculoviral transduction of human neurons

Zeng J, Du J, Lin J, Bak X, Wu C, Wang S (2009) High-efficiency transient transduction of human embryonic stem cell-derived neurons with baculoviral vectors. *Mol Ther* 17:1585–1592.

How can we improve the odds of cell therapy success? The transplantation of functional neurons derived from stem cells represents one of the great promises and challenges of cell therapeutics for the repair of central nervous system trauma and disease. Current technical challenges, including poor survival and inadequate functioning of transplanted cells, have limited the clinical success of this approach, and much work has gone into the development of genetic manipulation strategies designed to improve survival and function. One such strategy—the stable expression of transgenes that could enhance survival—has been shown to incur risks, including tumor formation and other detrimental effects. Therefore, transient transgene expression has attracted attention as a potential method for minimizing these risks.



Transgene expression in human neurons mediated by baculoviral vectors. (**a**) Phase contrast and fluorescence images of a group of live human neuron clusters transduced by baculoviral vectors carrying the expression cassette CMV.eGFP at a multiplicity of infection of 100 plaque-forming units per cell. The neurons were derived from HES-1. The pictures were taken 2 days after transduction. (**b–e**) Immunofluorescence staining showing the colocalization of neuronal markers (**b**) βIII tubulin, (**c**) MAP2ab, (**d**) NCAM, (**e**) NF200 with eGFP in HES-1–derived human neurons transduced by baculoviral vectors. (**f**) Immunofluorescence staining showing the colocalization of neuronal marker βIII tubulin and eGFP in human neurons derived from HES-3. The neurons were transduced by baculoviral vectors as described in (**a**). Bar = 500 µm (**a**); 200 µm (**b,f**); 100 µm (**c–e**). eGFP, enhanced green fluorescent protein; GFP, green fluorescent protein. Reprinted by permission from Macmillan Publishers Ltd: *Molecular Therapy* 17:1585 (2009).

In their current report, Zeng and colleagues describe the development of a baculovirus vector for transient gene transfer into human embryonic stem cell (hESC)–derived neurons, and demonstrate its effectiveness as a delivery vehicle using Green Fluorescent Protein (GFP) as a reporter. A transgene expression cassette that incorporated the CMV promoter was found to provide ~80% transfer efficiency; intense GFP expression was observed during the first week posttransduction, with substantial loss of expression observed in the second week. Addition of the posttranscriptional regulatory element WPRE from woodchuck hepatitis virus greatly extended the observed GFP expression, with intense fluorescence being visible as long as 12 weeks posttransduction. Transduction at 100 plaque-forming units per cell resulted in an 89% neuronal survival rate, demonstrating the inherent low cytotoxicity of baculoviral vectors. Further, the baculovirus-transduced neurons maintained transgene expression for as long as 4 weeks following their transplantation into the brains of nude mice, with no signs of physical abnormalities visible in the region of the graft.

These results suggest a potentially important role for baculoviral vectors in the development of efficient and clinically relevant cell therapy approaches. Learn more about these products at **www.invitrogen.com/bp61**. ■

Product	Quantity	Cat. No.
Bac-to-Bac® Baculovirus Expression System	20 reactions	A11100
Organelle Lights™ ER-OFP	1 kit	O36223
Organelle Lights™ Mito-OFP	1 kit	O36222
Alexa Fluor® 488–labeled rabbit anti-GFP antibody	100 µL	A21311

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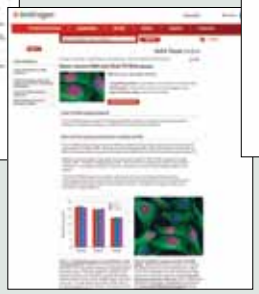

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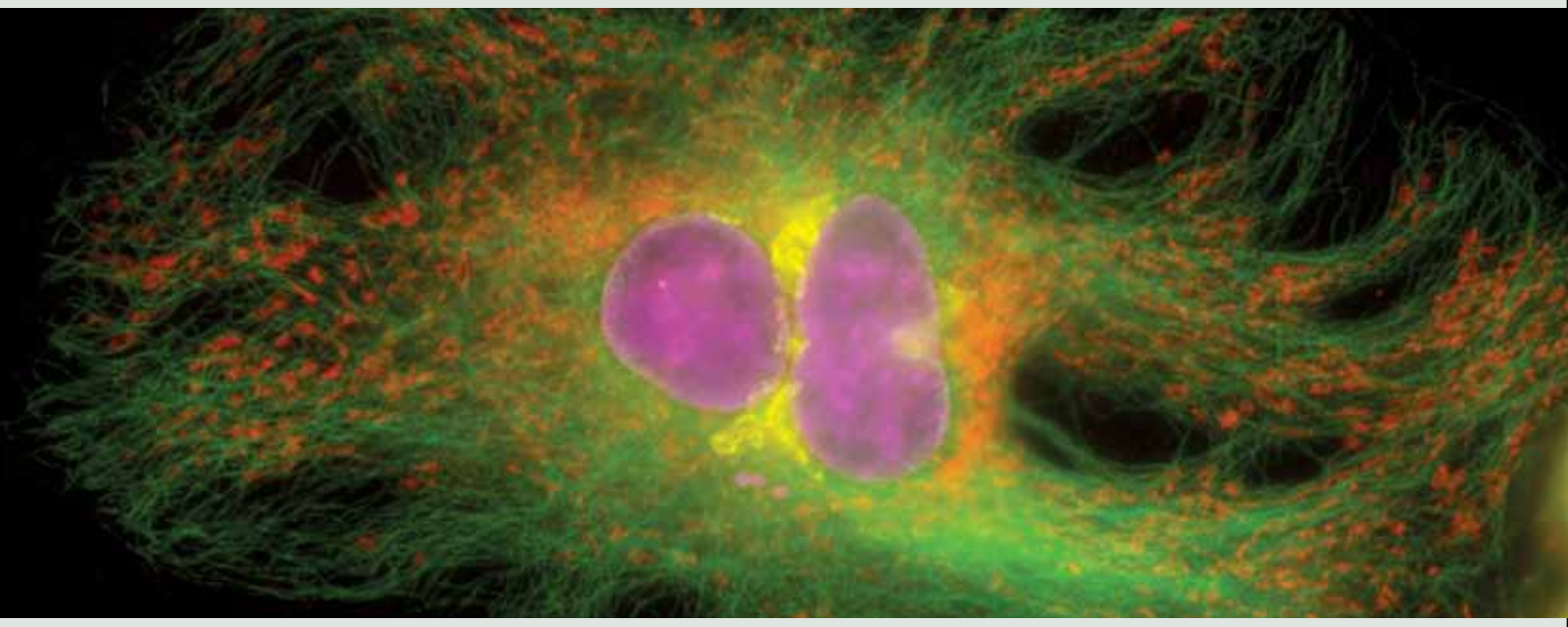
Environmental sustainability is an integral part of our overall mission to shape biological discovery and ultimately improve life. To this end, we have implemented numerous programs aimed at reducing our own environmental footprint, while focusing on providing products that help researchers to minimize their impact on the environment. Going beyond basic compliance, we incorporate design-for-environment principles at every stage in the product lifecycle. In September 2009, Life Technologies was again selected as a member of the Dow Jones Sustainability World Index, and ranked in the top 10% of the world's 2,500 largest companies in terms of sustainability. For details on these environmental sustainability efforts, download a copy of our 2008 Global Citizenship Report at **www.lifetechnologies.com/global-citizenship.html**. ■



Nanotechnology meets cellular analysis

QDOT® NANOCRYSTAL CONJUGATES IN FLOW CYTOMETRY AND IMAGING.

Fundamentally, Qdot® nanocrystals are fluorophores—substances that absorb photons of light, then reemit photons at a different wavelength. However, these particles fluoresce in a way that's completely different from traditional fluorophores. Their intrinsic brightness is often many times that observed for organic fluorophores, and their photostability is many orders of magnitude greater. These extraordinary fluorescence properties can be attributed to their unique structure and composition—Qdot® nanocrystals are nanometer-scale (roughly protein-sized) atomic clusters of semiconductor material. When conjugated to molecules such as primary or secondary antibodies or streptavidin, Qdot® nanocrystals provide exceptional fluorescence with full biofunctionality for a wide variety of life science applications, including flow cytometry and live-cell (Figure 1) and fixed-cell imaging. Studies requiring excellent photostability and multicolor detection from a single excitation source are likely to benefit from these unique bioconjugates.

Figure 1. Multiplex imaging of human carcinoma (HeLa) cell labeled with Qdot® nanocrystals and mounted with Qmount™ media. Mitochondria were detected with anti–OxPhos Complex V inhibitor protein IgG (Cat. No. A21355) and labeled using Qdot® 625 goat F(ab')$_2$ anti–mouse IgG (Cat. No. A10195, red fluorescence). The Golgi apparatus was detected with rabbit anti-giantin and labeled using Qdot® 585 goat F(ab')$_2$ anti–rabbit IgG conjugate (Cat. No. Q11411MP, yellow fluorescence). Tubulin was detected with rat anti-tubulin and labeled using DSB-X™ biotin goat anti–rat IgG (Cat. No. D20697) and Qdot® 525 streptavidin conjugate (Cat. No. Q10141MP, green fluorescence). Nuclei were labeled with Qnuclear™ Deep Red Stain (Cat. No. Q10363, purple fluorescence), and the slide was mounted using Qmount™ Qdot® Mounting Media (Cat. No. Q10336).

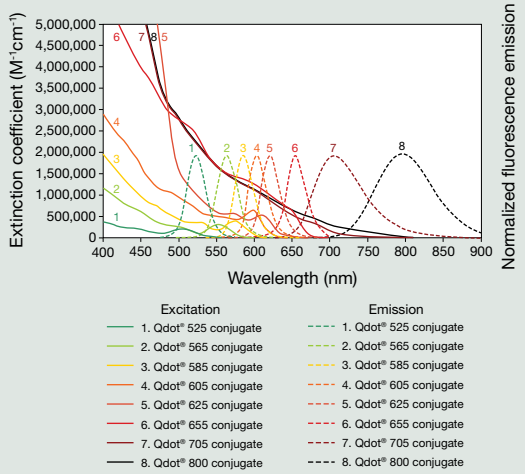


Figure 2. Extinction coefficients and emission profiles for selected Qdot® nanocrystal conjugates. Excitation is presented as extinction coefficient (left axis); emission is normalized to maximum peak height (right axis).

Why Qdot® nanocrystals?

Qdot® nanocrystals offer key advantages over traditional organic fluorochromes as fluorescent labels: they absorb light with efficiencies that are orders of magnitude beyond organic dye molecules, and they are extremely effective at converting this excitation energy into emitted photons. These two features make Qdot® nanocrystals much brighter than traditional organic fluorochromes.

The fluorescence properties of Qdot® nanocrystals are unique compared to those of typical dye molecules. Conventional fluorochromes such as fluorescein and R-phycoerythrin (RPE) have excitation and emission spectra with relatively small Stokes shifts, which means that the emission maximum is generally within 20–50 nm of the excitation maximum. Qdot® nanocrystals have symmetrical and relatively narrow emission peaks that can be 150–400 nm above their excitation wavelengths (Figure 2).

The unique ability of all Qdot® nanocrystals to be excited with the same wavelengths of light [1], optimally in the ultraviolet (UV) to blue range, enables multicolor labeling of cellular targets with minimal spectral overlap. Finally, unlike conventional dyes, Qdot® conjugates demonstrate exceptional photostability—they remain fluorescent under constant illumination, while conventional dyes photobleach to various extents.

Advantages of Qdot® nanocrystals for flow cytometry

Qdot® nanocrystal antibody conjugates allow the simultaneous analysis of 1 to 6 colors by flow cytometry and present the opportunity to replace problematic tandem dyes [2–5]. The nanocrystals are optimally excited by a UV or violet (405–407 nm) laser, but can also be excited, although with decreasing efficiency, by wavelengths lower than their emission wavelengths (Figure 2). Therefore, cross-laser, rather than within-laser, compensation must be carefully considered to account for spectral overlap.

In many cases, cross-laser spectral overlap is easily handled with filter selection. Although the emission profiles of the Qdot® →

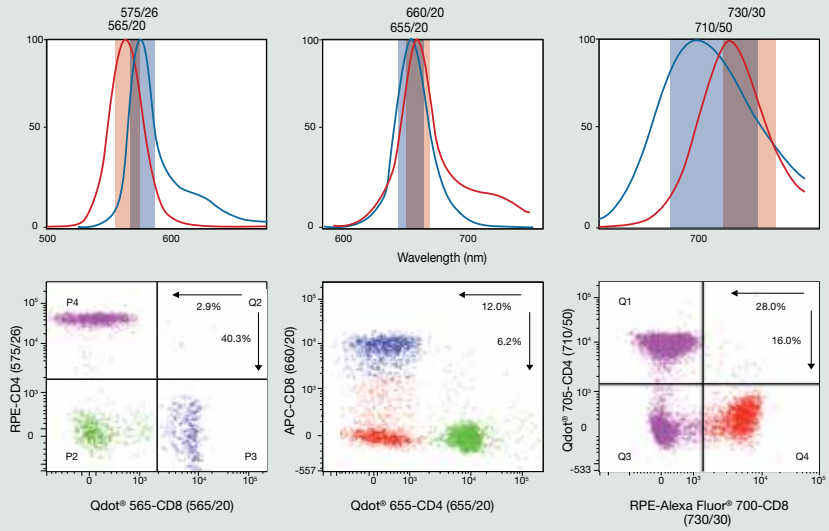


Figure 3. Filter selection for Qdot® nanocrystal and fluorescent dye combinations. Human peripheral blood leukocytes (PBLs) were stained with Qdot® nanocrystal and conventional dye (RPE, APC, and RPE-Alexa Fluor® 700) conjugates of anti-CD4 and anti-CD8. Samples were gated on lymphocytes by scatter and collected with the identified filters on instruments equipped with a 25 mW violet laser. Compensation matrices were calculated with single-color controls. Axes are labeled with the filters used; plots are labeled with compensation values.

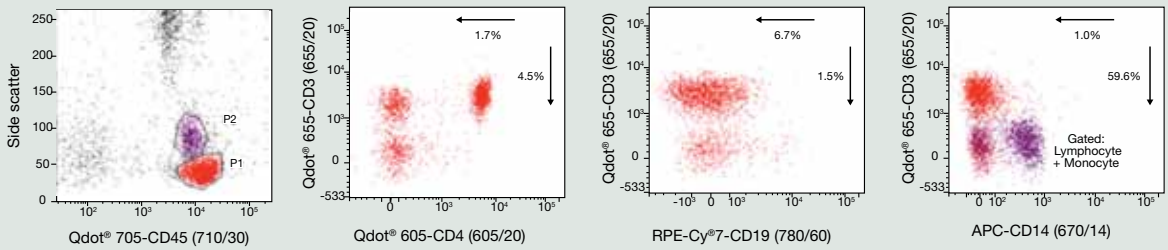


Figure 4. Eight-color immunostain combination with low compensation values. Human peripheral blood leukocytes (PBLs) were stained with Qdot® 605–anti-CD4, Qdot® 655–anti-CD3, Qdot® 705–anti-CD45, FITC–anti-CD2, RPE–anti-CD16+CD56, RPE-Cy®7–anti-CD19, APC–anti-CD14, and APC-Alexa Fluor® 750–anti-CD8 conjugates. Samples were run on a BD LSR II flow cytometer. Plots are gated on lymphocytes by side scatter/CD45. Axes are labeled with the filters used; plots are labeled with compensation values.

565 nanocrystal and RPE show considerable overlap (Figure 3), the nanocrystal is poorly excited by 488 nm and can be used with RPE with relatively little compensation. Similarly, the Qdot® 655 nanocrystal and allophycocyanin (APC) can be used together with conventional APC filters. The Qdot® 705 nanocrystal shows severe spectral overlap with Cy®5.5 RPE conjugates but can be used with Alexa Fluor® 700 RPE conjugates. Figure 4 shows selected plots from human peripheral blood leukocytes stained with an 8-color reagent combination. Through filter optimization and careful matching of antibodies to fluorophores, most compensation values can be maintained below 15%.

For flow cytometry, Qdot® nanocrystal antibody conjugates are generally used with violet laser excitation and analyzed with relatively narrow bandpass filters centered on their emission maxima (Table 1). For instruments with fixed-filter configurations such as the BD FACScan™ flow cytometer, specific nanocrystals can be matched to the filters installed on the instrument.

Compatibility with flow cytometry sample preparation reagents

Qdot® nanocrystals are compatible with most sample preparation reagents used in flow cytometry. Aldehyde-based fixatives will cause some decrease in Qdot® nanocrystal fluorescence and, of the fixatives tested, IC Fixation Buffer (Cat. No. FB01) provides the best preservation of Qdot® nanocrystal fluorescence. Reagents commonly used to permeabilize cells after fixation including FIX & PERM®, BD Cytoperm™, 0.1% saponin, 0.05% Triton® X-100, and methanol solutions, have not been shown to damage Qdot® conjugate fluorescence. Similarly, most erythrocyte lysis reagents have little impact on Qdot® nanocrystal fluorescence.

Table 1. BD LSR II flow cytometer filter configurations for detection of selected Qdot® nanocrystals.

Qdot® nanocrystal	Emission maximum (nm)	BD LSR II filter configuration	
Qdot® 565	565	555LP	565/20
Qdot® 605	605	570LP	605/20
Qdot® 655	655	640LP	655/20
Qdot® 705	705	690LP	710/50
Qdot® 800	800	750LP	780/60

Qdot® nanocrystals in cellular imaging

Due to their extreme brightness and photostability, Qdot® nanocrystals represent an exciting new set of tools for fluorescence microscopy that complement traditional organic dyes and fluorescent proteins. These characteristics make Qdot® nanocrystals optimal for long-term and multiplexed imaging and for sample archiving. Qdot® nanocrystals are easily incorporated into standard immunostaining protocols for cells and tissues. However, it is important to note that optimization must be

Table 2. Qdot® filter selection guide for imaging and microscopy.

Qdot® nanocrystal*	Emission (nm), FWHM†	Filter type (Cat. No.)		
		Chroma	Omega	Semrock
Qdot® 525	525	32010	XF301-2	QD525-A
Qdot® 565	565	32009	XF302-2	Contact Semrock
Qdot® 585	585	32008	XF303-2	Contact Semrock
Qdot® 605	605	32007	XF304-2	QD605-A
Qdot® 625	625	Contact Chroma	XF309-2	QD625-A
Qdot® 655	655	32012	XF305-2	QD655-A
Qdot® 705	705	32015	XF306-2	Contact Semrock
Qdot® 800	796	Contact Chroma	XF307-2	Contact Semrock

*Optimal excitation: UV–400 nm. † FWHM = full-width half-maximum.

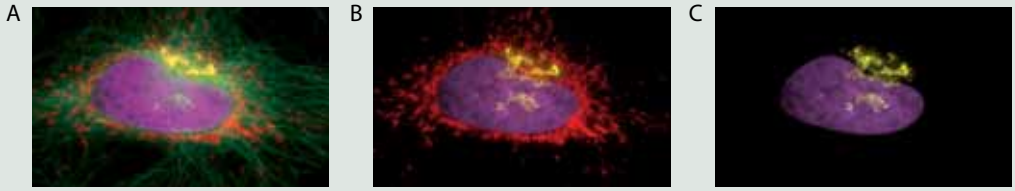


Figure 5. Multiplex cellular imaging of human carcinoma (HeLa) cell with Qdot® nanocrystal conjugates. The cell was labeled with mouse anti–OxPhos Complex V inhibitor protein IgG (Cat. No. A21355) and Qdot® 625–conjugated anti-mouse IgG (Cat. No. A10195) to visualize mitochondria (red), rabbit anti-giantin IgG and Qdot® 585–conjugated anti-rabbit IgG (Cat. No. Q11411MP) to visualize the Golgi complex, and rat anti-tubulin with biotinylated anti-rat IgG (Cat. No. D20697) and streptavidin–conjugated Qdot® 525 (Cat. No. Q10141MP) to stain the cytoskeleton (green). Nuclei (purple) were labeled with Qnuclear™ Deep Red Stain (Cat. No. Q10363), and samples were mounted in Qmount™ Qdot® Mounting Media (Cat. No. Q10336). (**A**) image showing all labels; (**B**) image excluding tubulin; (**C**) image excluding tubulin and mitochondria.

performed to achieve peak performance, even if organic dye–based conditions have already been determined [6]. Recommended filters for selected Qdot® nanocrystals are shown in Table 2.

Bright and photostable nuclear counterstaining

The new Qnuclear™ Deep Red Stain (Cat. No. Q10363) is a bright and photostable nuclear counterstain specifically designed for use with cells labeled with Qdot® 525, 565, 585, 605, 625, and 655 nanocrystals. With excitation and emission maxima of 640 and 663 nm, respectively, this counterstain is optimal for cell identification and multiplex imaging and can be visualized with standard fluorescence microscopy filter sets (Figure 5).

Mounting medium optimized for Qdot® fluorescence microscopy

Qdot® fluorescence is susceptible to chemical quenching, which represents a significant problem when using conventional mounting media. Invitrogen has recently developed a standardized mounting medium for Qdot®-labeled cells and tissue sections. Qmount™ Qdot® Mounting Media (Cat. No. Q10336) is a nonaqueous, permanent mountant designed for performing microscopy with Qdot® nanocrystals. Unlike other mountants, Qmount™ Qdot® Mounting Media causes no significant loss of the Qdot® nanocrystal fluorescence either initially or, more importantly, over the course of several months (Figure 6). Although optimal for use with Qnuclear™ Deep Red Stain, this mounting medium is not recommended for use with most standard organic dyes or fluorescent proteins.

The new Qdot® compatible nuclear counterstain and mounting reagent form a pair of important new tools that further enable the utility of Qdot® nanocrystal technology for fluorescence microscopy. Learn more about Qdot® technology and its applications at **www.invitrogen.com/bp61**. ■

FIX & PERM® is a registered trademark of An Der Grub Bio Research GmbH.

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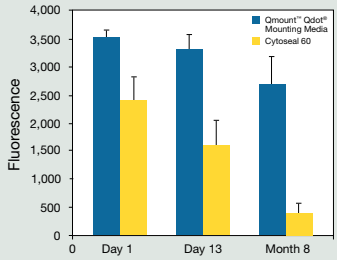

Figure 6. Fluorescence of Qdot®-labeled mammalian cells in Qmount™ Qdot® Mounting Media vs. Cytoseal 60. Human carcinoma (HeLa) cells were labeled with mouse anti–OxPhos Complex V inhibitor protein IgG (Cat. No. A21355) and Qdot® 605–conjugated goat anti–mouse IgG (Cat. No. Q11001MP), and mounted with Qmount™ Qdot® Mounting Media (Cat. No. Q10336) or Cytoseal 60 mountant. Cells were illuminated using a 100 W Hg-arc lamp and imaged on day 1, day 13, and month 8 after mounting, with identical image acquisition settings.

Novel recombinant monoclonal antibodies

ABFINITY™ TECHNOLOGY ENSURES PERFORMANCE.

ABfinity™ antibodies are highly specific, high-quality monoclonal antibodies that are unmatched in producing consistent results. Based on proprietary Invitrogen technology, ABfinity™ recombinant antibodies are developed by immunizing animals, screening for desired functionality, and then cloning the immunogen-specific antibody genes into high-level expression vectors. The antibodies are produced on a large scale by expressing them in mammalian cells, then highly purifying them with protein A. These recombinant antibodies can be used just like traditional IgG antibodies. The whole antibody is ~150 kDa as determined by nonreducing SDS-PAGE and, on a reducing gel, generates ~25 kDa light chain band and ~50 kDa heavy chain bands.

We offer a variety of ABfinity™ antibodies validated for various applications, including highly specific antibodies to AKT and other pathway members.

Lot-to-lot-consistency

ABfinity™ antibodies (Table 1) are manufactured by transfecting mammalian cells with heavy and light chain antibody cDNAs. This process is highly reproducible and results in unparalleled lot-to-lot consistency. This assurance of consistency saves time and money because assays do not require revalidation. Figure 1 shows the consistent western blotting results achieved using independent lots of an ABfinity™ antibody.

Reliable sensitivity and specificity

The ABfinity™ platform allows production of antibodies that are more sensitive and specific than those produced by other antibody development platforms. The high specificity of these antibodies ensures that

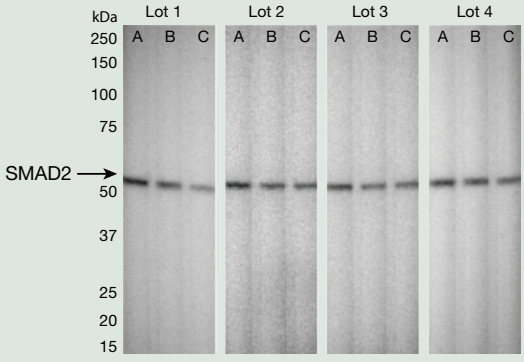

Figure 1. Lot-to-lot consistency of western blotting results with ABfinity™ antibody. HeLa cell extracts separated in reducing gels were probed with four different lots of Smad2 ABfinity™ Recombinant Rabbit Monoclonal Antibody (Cat. No. 700048). Concentrations of 2 µg/mL, 1 µg/mL, and 0.5 µg/mL of primary antibody were used in lanes A, B, and C, respectively. Primary antibodies were detected using the WesternBreeze® Chemiluminescent Kit–Anti-Rabbit (Cat. No. WB7106).

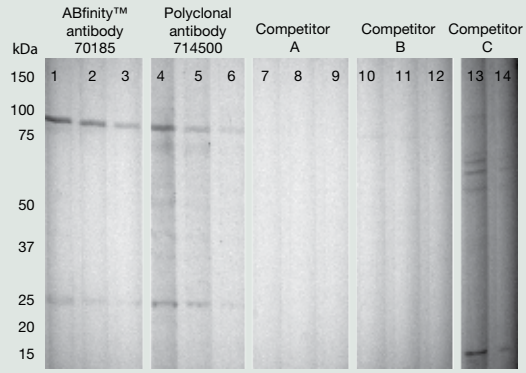

Figure 2. Superior western blotting results obtained using ABfinity™ antibody. The STAT4 ABfinity™ Recombinant Rabbit Monoclonal Antibody (Cat. No. 70185) and STAT4 Rabbit Polyclonal Antibody (Cat. No. 714500) were used at concentrations of 2 µg/mL, 1 µg/mL, and 0.5 µg/mL. STAT4 antibodies from other vendors were used at recommended concentrations. Primary antibodies were detected using the WesternBreeze® Chemiluminescent Kit–Anti-Rabbit (Cat. No. WB7106).

they only react with the target of choice, eliminating any detection of the wrong signal due to nonspecific binding. Highly sensitive antibodies can detect very low-level targets that may be difficult to detect with other antibodies. In addition, precious samples are saved by using less antibody for detection.

Figure 2 shows a direct comparison of an ABfinity™ STAT4 antibody with the best commercially available STAT4 antibodies. This comparison includes antibodies from polyclonal, traditional hybridoma monoclonal, and rabbit hybridoma monoclonal platforms.

Extensive validation and characterization

ABfinity™ antibodies are validated and characterized by multiple applications, including flow cytometry (Figure 3). This extensive validation process ensures that the antibodies can be used with confidence in target specificity and without any need for optimization.

Detecting the activated AKT kinase

PI3K/AKT is a cascading signaling pathway central to major cell functions, including cell growth, survival, apoptosis, angiogenesis, and the cell cycle.

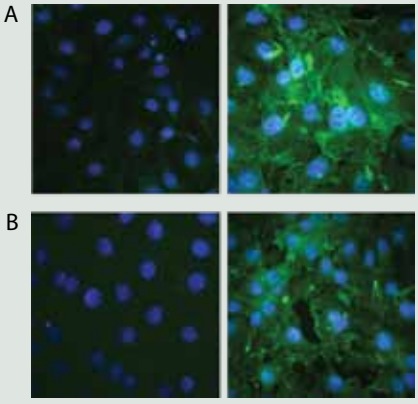

Figure 4. Immunocytochemistry analysis of mouse fibroblast cells labeled with AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody. (**A**) Mouse fibroblast cells were treated with (right) or without (left) 10 µg/mL insulin and labeled with AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody (Cat. No. 700392, 5 µg/mL). (**B**) For the insulin-treated cells in (**A**), the signal is knocked down after incubation with the phosphopeptide used as an immunogen (left) but not with the nonphosphopeptide (right). Alexa Fluor® 488 goat anti–rabbit IgG (Cat. No. A11008) at 1:1,000 was used as the secondary antibody. Nuclei were stained with one of the Hoechst dyes.

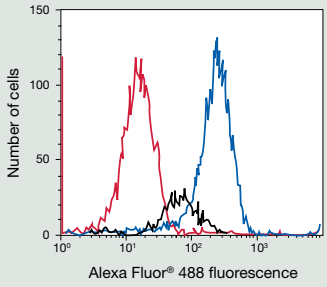

Figure 3. Flow cytometric detection of Jurkat cells labeled with JNK1/2 [pT183/pY185] ABfinity™ antibody. Jurkat cells were stimulated with 25 µg/mL anisomycin, then fixed and permeabilized using FIX & PERM® reagents (Cat. No. GAS-004). Cells were then stained with JNK1/2 antibody in the absence (blue trace) or presence (red trace) of the phosphopeptide immunogen, followed by detection using Alexa Fluor® 488 goat anti–rabbit IgG (Cat. No. A11008). The black trace represents unstimulated cells.

One of the main events in the PI3K/AKT pathway is phosphorylation of AKT at serine 473, resulting in activation of the kinase, which then acts on nuclear and cytoplasmic targets [1]. Detecting this phosphorylation event requires a very specific antibody that does not react with either unphosphorylated protein or protein phosphorylated at alternate sites. The AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody (Cat. No. 700392) recognizes the AKT protein only when it is phosphorylated at serine 473. The antibody has been validated by western blotting and can be used for rough quantification of the phosphorylated protein. Minute amounts of phosphorylated protein can be quantified using the AKT ABfinity™ antibody in a sandwich ELISA. The antibody has also been validated in immunocytochemistry and immunohistochemistry (Figures 4 and 5), allowing visualization of the cellular location of AKT in cultured cells (Figure 4) as well as in animal tissue (Figure 5). In addition, flow cytometry can be used to quantify phosphorylation at the cellular level and to separate phosphorylated and nonphosphorylated cell populations.

Other antibody tools for AKT signal transduction research

Additional ABfinity™ antibodies that have been developed for studying the effect of AKT signal transduction in glucose metabolism, the cell cycle, cell survival, adhesion, and angiogenesis are listed in Table 1. All of these antibodies are tested against multiple organisms and can be used in the applications listed in the table. We continue to offer one of the broadest portfolios of phosphorylation site–specific and total →

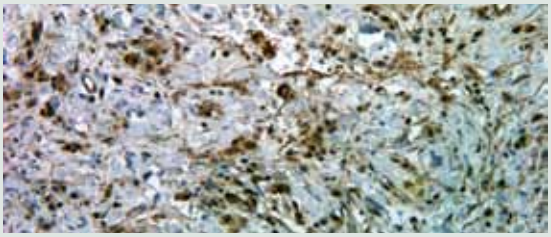

Figure 5. Immunohistochemistry analysis of human esophagus carcinoma tissue labeled with AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody. Formaldyehyde-fixed, parafin-embedded (FFPE) human esophagus carcinoma tissue was labeled with the AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody (0.5 µg/mL). The tissue was pretreated with EDTA and detected with SuperPicTure™ Polymer DAB (Cat. No. 87-8963). The image was taken at 20x magnification. Note nuclear and cytoplasmic staining in tumor cells.

antibodies. In addition, we test each lot of phosphorylation site–specific antibody by peptide competition to ensure that each lot of antibody detects only the protein phosphorylated at the correct site.

Take advantage of ABfinity™ antibody consistency

Highly specific, highly purified ABfinity™ recombinant antibodies are validated for a range of applications. For information about ABfinity™ antibodies against cell junction targets, see pages 18–19 of this issue. To view a complete list of validated Invitrogen antibodies, visit www.invitrogen.com/bp61. ■

Reference

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Table 1. ABfinity™ antibodies.

Description	Reactivity	Applications	Quantity	Cat. No.
Antibodies to AKT and other pathway members				
AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu (Z, X, Rt, Mk, Mk, Ma, Eq, Fe, Eq, Cp, Ch, Cn, B)	E, WB, F, IHC, IF/ICC	100 µg	700392
4E-BP1 [pT37] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Z, Rt, Ms, B, Eq)	E, WB, F, IHC, IF/ICC	100 µg	700238
AMPKβ1 [pS182] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (X, Rt, Or, Eq, Ch, Cn, B, Ms)	WB, F, IHC, IF/ICC	100 µg	700241
CASP3 [D175] ABfinity™ Recombinant Rabbit Monoclonal Antibody (clone 9H19L2)	Hu (X, Sw, Sh, Rt, Rb, P, Ms, Eq, Ha, Fe, Eq, Cp, Cn, B)	E, WB, F, IHC, IF/ICC	100 µg	700182
Cul-2 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Rt, Ms, Hu (X, Or, Mk, Eq, Cp, Cn, B)	WB, F, IHC, IF/ICC	100 µg	700179
IRAK4 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Sw, Sh, Rt, Qu, Eq, Cn, B)	F, IF/ICC	100 µg	700026
Mnk1 [pT197/pT202] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Z, X, Sw, Rt, P, Ms, Mk, Eq, Cp, Ch, Cn, B)	WB, F, IF/ICC	100 µg	700242
PKC-θ [pT538] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (X, Rt, Ms, Cp, B)	WB, F, IHC, IF/ICC	100 µg	700043
Pyk2 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Rt, Or, Ms, Mk, Eq, Cp, Cn, B)	F, IHC, IF/ICC	100 µg	700183
Smad1/5 [pS463/pS465] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Z, X, Sw, Sh, Rt, Ms, Mk, Eq, Cp, Ch, Cn, B)	F, IHC, IF/ICC	100 µg	700047
STAT4 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Rt, Ms, Hu (Sw, Eq)	E, WB, F, IHC, IF/ICC	100 µg	700185
SUMO-3 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu, Rt (Mk, Cp)	WB, F, IHC, IF/ICC	100 µg	700186
Other ABfinity™ antibodies				
Aβ [1-42] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu (Z, X, Sw, Rt, P, Or, Mk, Ha, Eq, Cp, Ch, Cn, B)	WB, IHC	100 µg	700254
AF-6 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu (Mk, Cp, Cn, B, Rt)	WB, IHC	100 µg	700193
Claudin-18 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu (Rt, Mk, Eq, Cp, Cn)	WB, IHC	100 µg	700178
c-Met ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (P)	WB, F, IF/ICC	100 µg	700261
ERK1/2 [pT185/pY187] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Z, X, Rt, Ms, Cp, Ch, B)	E, WB, IHC	100 µg	700012
IR/IGF1R [pY1162/pY1163] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (X, Rt, Ms, Mk, Cp, Ch, Cn, B, Eq)	E, WB	100 µg	700393
JNK1-2 [pTpY183/185] ABfinity™ Recombinant Rabbit Monoclonal Antibody (clone D12H7L17)	Hu (Z, X, Sw, Rt, Or, Ne, Ms, Mk, Gf, Eq, Cp, Ch, Cn, B)	E, WB, F, IHC, IF/ICC	100 µg	700031
PA28γ ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu, Rt (Z, X, Or, Eq, Ch, Cn)	WB, F, IHC, IF/ICC	100 µg	700180
Rab11 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (X, Rt, P, Ms, Eq, Ch, B)	WB, IF/ICC	100 µg	700184
Smad2 ABfinity™ Recombinant Rabbit Monoclonal Antibody (clone 31H15L4)	Hu (Z, X, Rt, Or, Ms, Gf, Ch, B)	E, WB, F, IF/ICC	100 µg	700048
T-bet ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Cp, Mk)	WB, F, IHC, IF/ICC	100 µg	700059

Reactivity: B = bovine; **Ch** = chicken; **Cn** = canine; **Cp** = chimpanzee; **Eq** = equine; **Fe** = feline; **Gf** = goldfish; **Ha** = hamster; **Hu** = human; **Ma** = mammalian; **Mk** = monkey (rhesus); **Ms** = mouse; **Ne** = nematode; **Or** = orangutan; **P** = primate; **Qu** = quail; **Rb** = rabbit; **Rt** = rat; **Sh** = sheep; **Sw** = swine; **X** = *Xenopus*; **Z** = zebrafish. **( )** indicates reactivity predicted but not tested. **Applications: E** = ELISA; **F** = flow cytometry; **ICC** = immunocytochemistry; **IF** = immunofluorescence; **IHC** = immunohistochemistry; **IP** = immunoprecipitation; **WB** = western blotting.

Single-sample detection of β-galactosidase and firefly luciferase in cell lysates

NOVABRIGHT™ CHEMILUMINESCENT DUAL REPORTER GENE ASSAY.

The NovaBright™ β-Galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection Kit is designed for the rapid and sensitive assay of firefly luciferase and β-galactosidase in the same sample. The use of two reporters, one as an experimental reporter (here, luciferase) and the other as a constitutively expressed transfection control reporter (β-galactosidase), is common and often necessary to accurately quantitate activity from experimental reporter constructs. In the NovaBright™ dual reporter gene assay, luciferase reporter enzyme activity is quantitated via an enhanced luciferase reaction; following a 30–60 minute incubation and addition of a light emission accelerator, β-galactosidase reporter enzyme activity is determined with Galacton-Plus® substrate (Figure 1). The entire assay is completed in less than one hour. Greater convenience and precision are obtained by combining both assays into one simple microplate assay.

Detection with high sensitivity and wide dynamic range

The NovaBright™ β-Galactosidase and firefly luciferase dual assay is 1,000-fold more sensitive than colorimetric reporter gene assays and has a wide dynamic range of over 7 orders of magnitude (femtograms to nanograms). The high sensitivity enables the detection of low levels of reporter enzyme produced by weak promoters or poorly transfected cells. The wide dynamic range allows measurement over a broad range of concentrations without the need for multiple sample dilutions.

Reliable quantitation in a variety of applications

The NovaBright™ dual reporter gene assay has been very widely used for reporter quantitation and transfection normalization of transiently transfected mammalian cell lines [1–5] as well as transfected primary cells [6–9]. For more information on how the assay can be used in such applications, visit **www.invitrogen.com/bp61**. ■

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Product	Quantity	Cat. No.
NovaBright™ β-Galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection Kit	200 microplate assays	N10561
	600 microplate assays	N10562

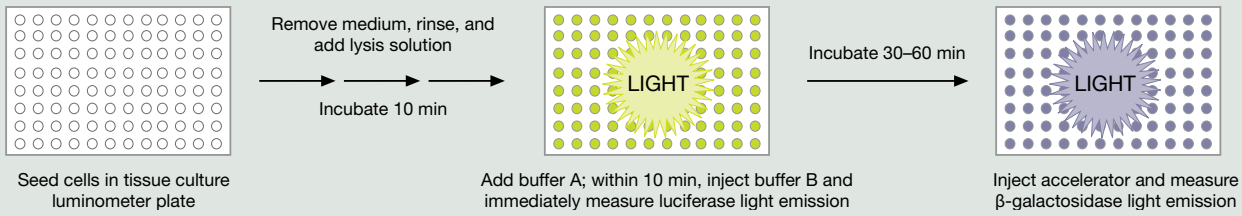

Figure 1. Sequential dual assay of samples for firefly luciferase and β-galactosidase using the NovaBright™ kit.

Modeling angiogenesis *in vitro*

CELL PROLIFERATION AND ENDOTHELIAL TUBE FORMATION ASSAYS.

Angiogenesis—the formation of new blood vessels from existing vasculature—is an integral part of both normal and pathological processes. During angiogenesis, endothelial cells disrupt the surrounding basement membrane, migrate toward an angiogenic stimulus, proliferate to provide additional cells that will form the new vessel, and reorganize to create the necessary three-dimensional vessel structure. *In vitro* assays are widely used to study these functions in the presence of either angiogenic or antiangiogenic agents.

Click-iT® EdU cell proliferation assays offer accurate measurement of endothelial cell proliferation. We also recently developed a new protocol for one of the most well-established assays to model the formation of three-dimensional vessels, the endothelial tube formation assay.

Endothelial cell proliferation studies

The most reliable cell proliferation assays detect cell division by directly measuring DNA synthesis. The thymidine incorporation assay is the most common method, wherein the radioactive nucleoside [^{3}H] thymidine is incorporated into new strands of chromosomal DNA during the S phase of the cell cycle. This proliferation assay is laborious and slow, offers poor resolution of sample structure, and suffers from all the potential health and waste-disposal concerns inherent in radioligand methodology.

More recently developed assays, such as BrdU labeling, have eliminated the need to use radioisotopes [1], but the success of this approach necessitates extensive sample denaturation that can be difficult to reproduce and can significantly degrade sample structure.

To address this challenge, Click-iT® EdU cell proliferation assays employ the nucleoside analog EdU (5-ethynyl-2′-deoxyuridine) and a detection method that is not antibody-based and therefore does not require DNA denaturation. Click-iT® EdU detection is faster, easier, and more reliable than BrdU assays. Regardless of the cell proliferation assay used, it should be noted that the inhibition of endothelial cell proliferation is not necessarily evidence of an anti-angiogenic effect. In some cases, the angiogenic test agent may simply be toxic to the cells, and results should be confirmed using additional assays.

Detecting endothelial cell reorganization

The tube formation assay is widely used to model the reorganization stage of angiogenesis. This *in vitro* assay measures the ability of endothelial cells, plated at subconfluent densities with the appropriate extracellular matrix support, to form capillary-like structures (known as tubes). This assay is typically used to determine the ability of various

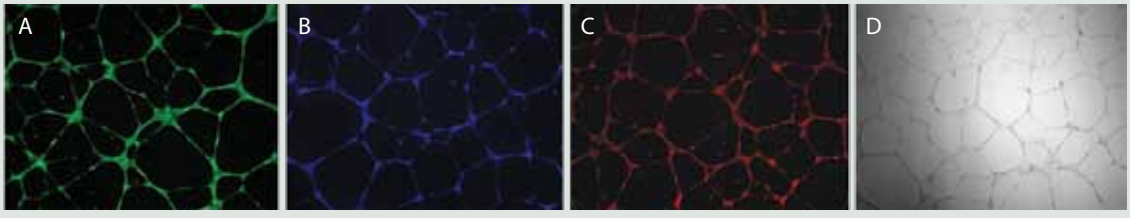

Figure 1. Induction of endothelial cell reorganization into 3D vessel structures. Human umbilical vein endothelial cells (HUVEC) (42,000 viable cells/cm^2) were seeded on a 24-well polystyrene plate coated with Geltrex™ matrix (50 µL/cm^2) using LSGS-supplemented Medium 200PRF, and incubated at 37°C and 5% CO_2. At 16 hr post-seeding, 2 µg/mL of (**A**) Calcein, AM (Cat. No. C3099), (**B**) Calcein Blue, AM (Cat. No. C1429), or (**C**) CellTrace™ Calcein Red-Orange, AM (Cat. No. C34851) was added directly to the culture well and incubated for 20 min (37°C, 5% CO_2) prior to imaging at 4x magnification. (**D**) A representative phase-contrast image.

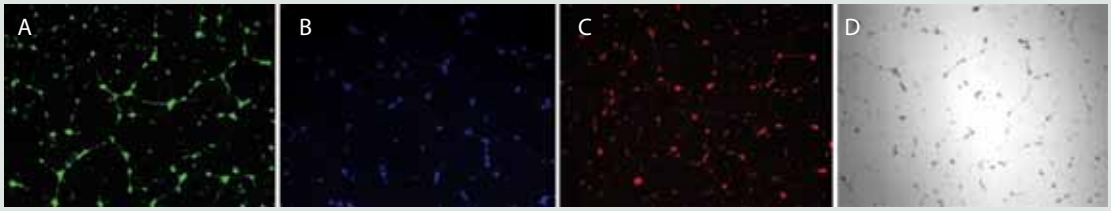

Figure 2. Low background of the endothelial tube formation assay. Human umbilical vein endothelial cells (HUVEC) (42,000 viable cells/cm^2) were seeded on a 24-well polystyrene plate coated with Geltrex™ matrix (50 µL/cm^2) using non-supplemented Medium 200PRF, and incubated at 37°C and 5% CO_2. At 16 hr post-seeding, 2 µg/mL of (**A**) Calcein, AM (Cat. No. C3099), (**B**) Calcein Blue, AM (Cat. No. C1429), or (**C**) CellTrace™ Calcein Red-Orange, AM (Cat. No. C34851) was added directly to the culture well and incubated for 20 min (37°C, 5% CO_2) prior to imaging at 4x magnification. (**D**) A representative phase-contrast image.

compounds to promote or inhibit tube formation. Compounds that inhibit tube formation are potentially useful for treating various diseases such as cancer, where tumors stimulate new blood vessel formation to receive oxygen and nutrients for growth.

Upon plating, endothelial cells attach to and generate mechanical forces on the surrounding extracellular support matrix to create guidance pathways or "tracks" that facilitate cellular migration. The resulting cords of cells will eventually form hollow lumens. Once formed, these interconnected networks are usually maintained for approximately 24 hours. Tube formation is typically quantified by measuring the number, length, or area of these capillary-like structures in two-dimensional microscope images of the culture dish.

We recently developed a detailed protocol designed to promote the formation of endothelial cell tube networks using human umbilical vein endothelial cells (HUVEC) and Geltrex™ Reduced Growth Factor Basement Membrane Matrix, with an optional step for fluorescent monitoring of tube formation using a cell-permeable dye (e.g., calcein, AM). Figure 1 demonstrates the successful formation of endothelial tube networks using this protocol. As shown in Figure 2, there is no significant tube formation in the absence of an angiogenic inducer, demonstrating low background and thus a high signal-to-noise ratio using these cells and reagents. The endothelial tube formation assay protocol is available for download at **http://tools.invitrogen.com/content/sfs/manuals/Endothelial_Cells_Tube_Formation.pdf**.

The advantages of the endothelial tube formation assay include its relatively easy setup, short culture period, quantifiable results, and high-throughput capabilities. In addition, stringent manufacturing protocols for HUVEC and Geltrex™ matrix ensure consistent and reliable data. Each lot of Geltrex™ Reduced Growth Factor Matrix is functionally tested for tube formation, ensuring the highest performance and lot-to-lot consistency.

While the tube formation assay is essential for the study of endothelial reorganization, results should be confirmed *in vivo*, because commercially available endothelial cells have been preselected for their proliferative capacity and heterospecific cell interactions are not represented. It has also been reported in the literature that certain non-endothelial cell types demonstrate tube formation, which suggests that tube formation by endothelial cells *in vitro* may not represent true differentiation of this cell type [2].

Advance your angiogenesis research

We offer a diverse selection of antibodies, proteins, and assays for the analysis of angiogenesis. Learn more at **www.invitrogen.com/bp61**. ■

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Product	Quantity	Cat. No.
Cell proliferation assays		
Click-iT® EdU Alexa Fluor® 488 Imaging Kit	1 kit	C10337
Click-iT® EdU Alexa Fluor® 555 Imaging Kit	1 kit	C10338
Click-iT® EdU Alexa Fluor® 594 Imaging Kit	1 kit	C10339
Click-iT® EdU Alexa Fluor® 647 Imaging Kit	1 kit	C10340
Tube formation assay reagents		
Calcein, AM, 1 mg/mL solution in anhydrous DMSO	1 mL	C3099
Cryopreserved Primary Human Umbilical Vein Endothelial Cells (HUVEC)	≥500,000 viable cells	C-003-5C
Geltrex™ Reduced Growth Factor Basement Membrane Matrix	5 mL	12760-021
Low Serum Growth Supplement (LSGS)	10 mL	S-003-10
Medium 200PRF	500 mL	M-200PRF-500
Trypsin/EDTA Solution (1X)	100 mL	R-001-100
Trypsin Neutralizer Solution (1X)	100 mL	R-002-100

Multiparametric approaches for predictive hepatotoxicity

COMBINING ASSAYS FOR MITOCHONDRIAL DYSFUNCTION, MEMBRANE INTEGRITY, AND OXIDATIVE STRESS.

Hepatotoxicity is one of the major causes of candidate drug attrition in preclinical and clinical drug development. Implementation of cell-based multiparametric assays for hepatotoxicity early in the drug discovery process can provide predictive information and also serves as a cost-effective complement to, or even replacement for, later-stage animal testing. Mitochondrial dysfunction, compromise of plasma membrane integrity, and oxidative stress may all be exploited as reliable markers for cell-based hepatotoxicity assays. The assays described here are robust in the HepG2 cell model and offer excellent multiparametric approaches for predictive hepatotoxicity.

Quantitative assessment of mitotoxicity and cytotoxicity

Mechanisms of drug-induced hepatotoxicity often involve the depolarization of the inner mitochondrial membrane and, ultimately, cell death, characterized by loss of plasma membrane integrity. Thus, the loss of mitochondrial membrane potential and plasma membrane integrity may serve as early and late biomarkers, respectively, for drug-related hepatotoxicity [1–6].

The HCS Mitochondrial Health Kit was developed for simultaneous, quantitative measurements of these two parameters of cell health by high-content analysis (Figure 1). The MitoHealth Stain accumulates in mitochondria in proportion to the mitochondrial membrane potential; loss of membrane potential is visualized by a decrease in staining intensity. Cytotoxicity is measured in the same cells with the membrane-impermeant Image-iT® DEAD Green™ Viability Stain. This stain is permeant only to cells with compromised plasma membranes, has high affinity for DNA, and fluoresces only when bound to DNA. Thus, DNA staining is used as an indicator of cytotoxicity.

Both stains retain fluorescence upon formaldehyde fixation and detergent-based permeabilization, allowing for multiplexing with antibody labeling. Hoechst 33342 is also included as a nuclear segmentation tool and as an indicator of cell loss.

Cell-based measurements of oxidative stress

Reduced glutathione (GSH) plays an important role in protecting cells against oxidative stress and toxicity. GSH represents the majority of free

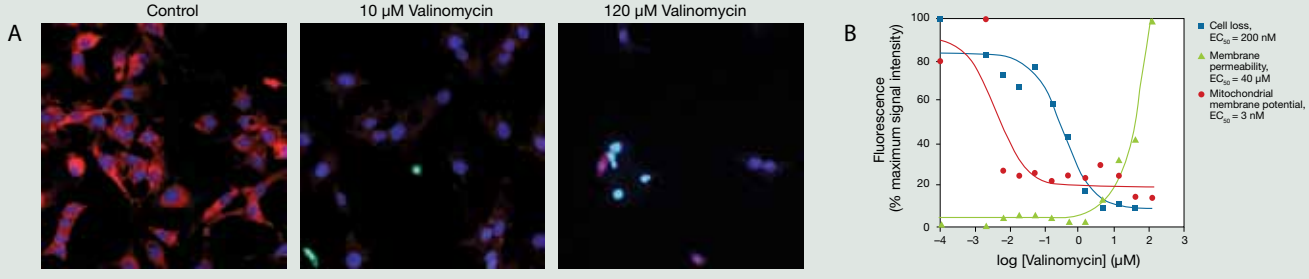

Figure 1. Measuring mitochondrial membrane potential and plasma membrane integrity simultaneously with the HCS Mitochondrial Health Kit. HepG2 cells were plated on collagen I–coated plates, treated with various concentrations of valinomycin for 24 hr, and stained with Image-iT® DEAD Green™ Viability Stain and MitoHealth Stain for 30 min. Cells were then fixed and counterstained with Hoechst 33342 nuclear stain. The Thermo Scientific Cellomics ArrayScan® VTI was used for imaging and analysis. (**A**) Images at selected concentrations of valinomycin. Loss of mitochondrial membrane potential is reflected by a decrease in MitoHealth staining (red). Loss of plasma membrane integrity is visualized by an increase in Image-iT® DEAD Green™ Viability Stain fluorescence (green). The loss of Hoechst staining reflects a reduction in cell number. (**B**) Logs of valinomycin concentrations plotted against 3 parameters: cell loss (blue), membrane permeability (green), and mitochondrial membrane potential (red). EC_{50} values were calculated using percent maximum values.

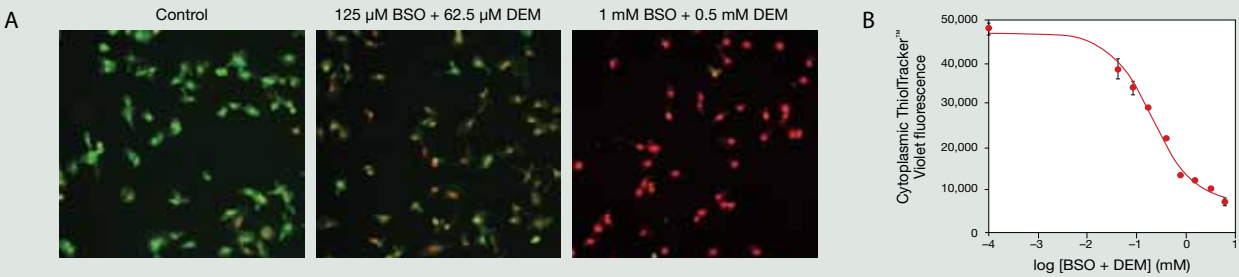

Figure 3. High-content imaging and analysis of reduced glutathione (GSH) in HepG2 cells. Cells were plated on collagen-coated 96-well plates and treated with 2-fold dilutions of buthionine sulfoximine (BSO, 4 mM highest dose) and diethyl maleate (DEM, 2 mM highest dose) in combination for 2 hr. Cells were stained with 20 µM ThiolTracker™ Violet (green) and HCS NuclearMask™ Deep Red (red) for 30 min in thiol-free buffer. Cells were then washed once with buffer and imaged on the Thermo Scientific Cellomics ArrayScan® VTI. (**A**) Images at selected concentrations of BSO and DEM. A decrease in GSH is indicated by a reduction in ThiolTracker™ Violet fluorescence. (**B**) Cytoplasmic intensity of ThiolTracker™ Violet fluorescence plotted against log (BSO + DEM) concentration. The combined EC_{50} value of BSO and DEM is 196 µM.

thiols in healthy cells, and a decrease in GSH levels is a reliable indicator of oxidative stress. ThiolTracker™ Violet, a bright fluorescent thiol-reactive dye, can be used to measure GSH in cells using fluorescence microscopy and high-content imaging [7,8].

ThiolTracker™ Violet reacts with GSH, generating an extremely bright GSH derivative. ThiolTracker™ Violet–labeled GSH is much brighter than monochlorobimane (mBCl), traditionally used for fluorescence-based GSH measurements. This difference defines ThiolTracker™ Violet as the probe of choice in imaging applications, as the bimane-based probes, including mBCl, are typically too dim for visualizing changes in GSH levels by fluorescence microscopy (Figure 2).

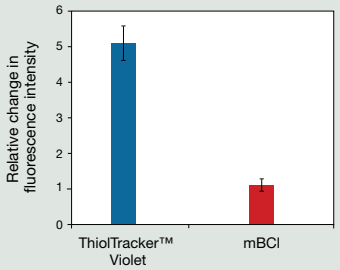

Figure 2. Greater fluorescence intensity of ThiolTracker™ Violet compared to mBCl for detection of reduced glutathione. HepG2 cells were plated on collagen-coated plates and then treated with 4 mM buthionine sulfoximine (BSO) and 2 mM diethyl maleate (DEM) for 2 hr. Cells were stained with either 20 µM ThiolTracker™ Violet or 80 µM mBCl for 30 min. The cells were imaged and analyzed using the Thermo Scientific Cellomics ArrayScan® VTI. The ratio of total cellular intensities of ThiolTracker™ Violet and mBCl in control samples compared to treated samples is shown.

ThiolTracker™ Violet staining is not amenable to fixation or detergent permeabilization—the dye is directly applied to live cells in a thiol-free buffer or complete medium. Cells can be directly imaged after staining and washing, using a 405 nm or 488 nm laser or with traditional xenon or mercury arc lamps using standard DAPI/FITC filter sets. HCS NuclearMask™ Deep Red is an ideal segmentation tool for high-content imaging in combination with ThiolTracker™ Violet dye (Figure 3).

Cell-based assays for predictive hepatotoxicity

Mitochondrial dysfunction, membrane integrity, and reduced glutathione levels are reliable early markers of hepatotoxicity and, when combined with other biomarkers, can serve as important components of cell-based approaches for increasing the predictive value of toxicity screens. For more information, visit **www.invitrogen.com/bp61**. ■

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Product	Quantity	Cat. No.
HCS Mitochondrial Health Kit	2 plates	H10295
Image-iT® DEAD Green™ Viability Stain	25 plates	I10291
ThiolTracker™ Violet Dye	5 plates	T10096
HCS NuclearMask™ Deep Red	10 plates	H10294

Tools for induced pluripotent stem cell research

GIBCO® MEDIA AND REAGENTS, AND TAQMAN® GENE EXPRESSION ASSAYS.

The relatively new discovery that pluripotent human cells can be derived from somatic cells through the expression of exogenous genes has generated great excitement and promise in the fields of biology and cell therapy [1–5]. Unlike embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) do not present any cultural or ethical barriers for use, are derived from somatic cells that are plentiful, and can potentially overcome immune rejection issues when used for cell therapy.

Our broad technology platform provides optimized tools for every aspect of iPSC research—from isolation and reprogramming of somatic cells to validation and differentiation of iPSCs. Here we present methods for selection and expansion of iPSCs and for validating iPSC identity using TaqMan® Gene Expression Assays. Tools for the isolation, expansion, and reprogramming of somatic cells will be discussed in an upcoming issue.

Selection and expansion of iPSCs

GIBCO® media and reagents have been at the forefront of ESC research for years, and now they are an integral part of iPSC research. With our flexible offering of media systems, iPSCs can be efficiently isolated and expanded with or without feeder cells or animal components.

Traditional culture conditions for ESCs and iPSCs are well established [2,3] and usually require cells to be grown on a mitotically inactive feeder cell layer. The medium generally recommended is a DMEM formulation supplemented with FBS. KnockOut™ SR is a defined serum-free formulation designed to directly replace FBS in ESC and iPSC cultures, and provides more consistent growth conditions (Figure 1A).

More recently, scientists have begun to move away from culture methods using serum and feeder layers. These methods are labor-intensive and hard to scale, and their inherent variability makes it difficult to maintain iPSCs in an undifferentiated state. StemPro® hESC SFM, originally developed for the serum- and feeder-free culture of human embryonic stem cells (hESCs), provides a breakthrough solution to these problems by enabling serum- and feeder-free culture of human iPSCs (Figure 1B). The cGMP-manufactured components of StemPro® hESC SFM give you the quality and consistency needed to optimize your iPSC culture.

Animal-derived components in culture media and animal-origin feeder cells can contaminate human iPSCs and ESCs with animal pathogens. Facilitating the transition of human iPSCs from the lab to clinical applications, KnockOut™ SR XenoFree is the first xeno-free medium for human iPSC culture (Figure 1C). KnockOut™ SR XenoFree can be adapted for use with or without human feeder cells by using the CELLstart™ humanized substrate for cell culture.

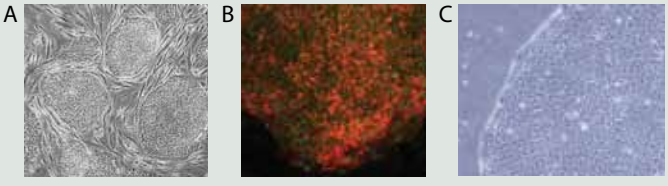
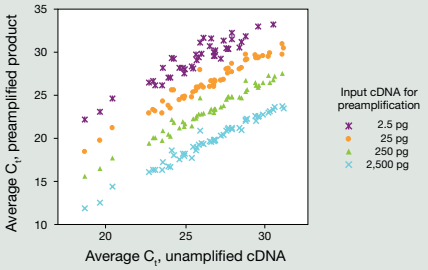


Figure 1. iPSCs cultured in feeder-based (A), feeder-free (B), and xeno-free (C) media systems. (**A**) Phase-contrast image of iPSCs grown in KnockOut™ SR. (**B**) Oct-4/Tra-1-81 staining of human iPSCs grown in StemPro® hESC SFM, confirming pluripotency. Data provided by Jeanne Loring, Scripps Research Institute. (**C**) Human iPSCs derived, expanded, and grown to passage 5 in KnockOut™ SR XenoFree without feeder cells using CELLstart™ xeno-free substrate and KnockOut™ SR XenoFree Growth Factor Cocktail. Data provided by Hidenori Akutsu, National Center for Child Health and Development, Tokyo, Japan.

Validating iPSC identity using TaqMan® Gene Expression Assays

Gene expression profiling can be used to validate the expression of specific genes associated with stem cell identity. The profiling of ESCs and iPSCs requires technology that is compatible with very small samples, yet is capable of analyzing the expression of many mRNAs simultaneously. Using the Applied Biosystems® TaqMan® PreAmp Cells-to-CT™ Kit, cDNA can be prepared directly from cultured cells, even at the single cell



Method	Input cDNA per target set	# Cell equivalents	r^2	# Targets detected	% Targets detected
Preamplification	2.5 pg	0.1	0.89	47	78
	25 pg	1	0.96	56	93
	250 pg	10	0.97	55	92
	2.5 ng	100	0.97	56	93
No preamplification	200 ng	10,000	1.00	60	100

Figure 2. Linearity of cDNA preamplification in mouse embryonic stem cells (mESCs). Following reverse transcription, multiplex preamplification of cDNA was performed using the TaqMan® PreAmp Pool for Mouse Stem Cell Pluripotency Array and TaqMan® PreAmp Master Mix, and cDNA was preamplified for 14 cycles. The product was then diluted and combined with TaqMan® Gene Expression Master Mix, and real-time PCR was performed in duplicate using theTaqMan® Mouse Stem Cell Pluripotency Array. Preamplification provides results similar to unamplified cDNA, while using 10,000-fold less input.

level (Figure 2). The cDNA is first preamplified using TaqMan® PreAmp Master Mix and TaqMan® PreAmp Pools, a mixture of gene-specific PCR primers. Gene expression analysis is then carried out using singleplex real-time PCR with TaqMan® Human or Mouse Stem Cell Pluripotency Arrays, which contain real-time PCR assays for a defined set of validated human ESC identity markers and 6 endogenous controls, in an easy-to-use microfluidic card format. Arrays are run on the Applied Biosystems® 7900HT Fast Real-Time PCR System (Figure 3) [6]. ■

Complete solutions for iPSC research

GIBCO® media, reagents, and substrates and TaqMan® Gene Expression Assays are reliable and efficient tools for the isolation, expansion, and validation of iPSCs. For more information on the complete range of reagents available for iPSC research, including our extensive menu of growth factors for iPSC differentiation, visit **www.invitrogen.com/bp61**. ■

Accutase® is a registered trademark of Innovative Cell Technologies.

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Product	Quantity	Cat. No.
iPSC isolation and expansion		
KnockOut™ Serum Replacement	500 mL	10828-028
KnockOut™ DMEM	500 mL	10829-018
StemPro® hESC SFM	1 kit	A1000701
KnockOut™ SR XenoFree	500 mL	A1099202
KnockOut™ SR XenoFree Growth Factor Cocktail	10 mL	0090003SA
Fetal Bovine Serum, ES Cell-Qualified	500 mL	10439024
Recombinant Human LIF	100 µg	PHC9461
CELLstart™ substrate	2 mL	A10142-01
Geltrex™ hESC-Qualified RGF Basement Membrane Matrix	1 mL	A1048001
Geltrex™ hESC-Qualified RGF Basement Membrane Matrix	5 mL	A1048002
FGF-basic, Recombinant Human	10 µg	PHG0026
	100 µg	PHG0021
	1 mg	PHG0023
StemPro® EZPassage™ Disposable Stem Cell Passaging Tool	10 units	23181010
StemPro® Accutase® Cell Dissociation Reagent	100 mL	A11105-01
TrypLE™ Express Stable Trypsin-Like Enzyme With Phenol Red	100 mL	12605-010
TaqMan® Gene Expression Assays		
TaqMan® PreAmp Cells-to-CT™ Kit	40 reactions	4387299
TaqMan® PreAmp Pool for Human Stem Cell Pluripotency Array	65 reactions	4405625
TaqMan® Human Stem Cell Pluripotency Array	4 cards	4385344
TaqMan® Array Human Stem Cell Pluripotency Plate	96 reactions	4414077
TaqMan® PreAmp Pool for Mouse Stem Cell Pluripotency Array	65 reactions	4405626
TaqMan® Mouse Stem Cell Pluripotency Array v.2	4 cards	4385363
TaqMan® Array Mouse Stem Cell Pluripotency Plate	96 reactions	4414080

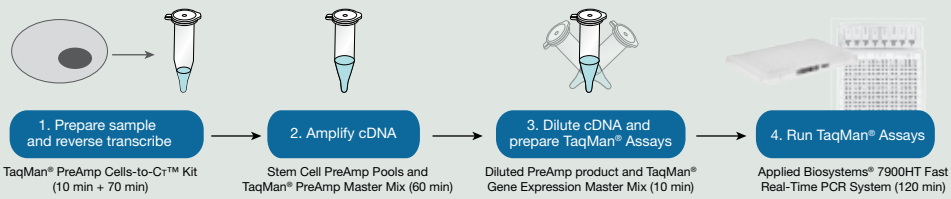

Figure 3. TaqMan® PreAmp real-time PCR workflow for single-cell expression profiling.

Highly specific antibodies for cell junction studies

VALIDATED ANTIBODIES FOR CELL JUNCTION TARGETS.

Cell junctions play a key role in regulating small-molecule trafficking between cells, organizing cells into tissues, and adhering cells to each other and to the extracellular matrix. Dysfunctional junctions have been implicated in several pathophysiological conditions, including cancer, neurodegeneration, and cardiovascular dysfunction, among others. The five main types of cell junctions are shown in Figure 1.

Conjugates for a broad range of targets

High-quality antibodies to cell junction targets have been conjugated to Alexa Fluor® dyes—the brightest and most trusted fluorescent dyes available (Table 1). These conjugates are useful not only for direct conjugation but also for colabeling with other antibodies. The sensitivity afforded by the brightness of these antibodies approaches the sensitivity of detection with a secondary antibody detection system. Figure 2 demonstrates the excellent antibody specificity in visualizing tight junctions between human epithelial colorectal adenocarcinoma cells.

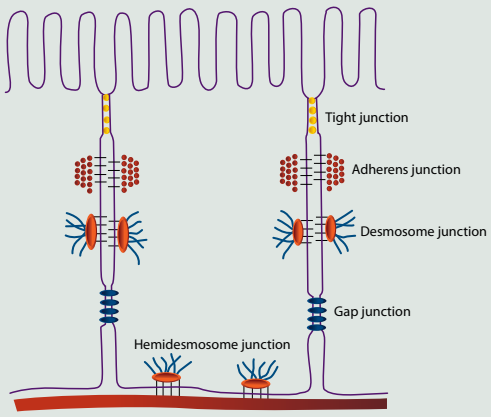

Figure 1. The five main types of cell junctions.

Validation with multiple applications

We offer one of the most extensive collections of antibodies for cell junction studies, covering all five primary types of cell junctions: gap junctions, tight junctions, adherens junctions, desmosomes, and hemidesmosomes (Table 2). These antibodies have been validated for multiple applications, including western blotting, ELISA, immunohistochemistry, immunocytochemistry, and immunoprecipitation.

Recombinant ABfinity™ antibodies for even higher specificity

ABfinity™ antibodies are the next generation of antibodies, presented exclusively by Invitrogen. These antibodies are generated by cloning the specific antibody genes and producing them in a mammalian expression system. ABfinity™ technology brings you the most specific antibodies available, resulting in highly reproducible data.

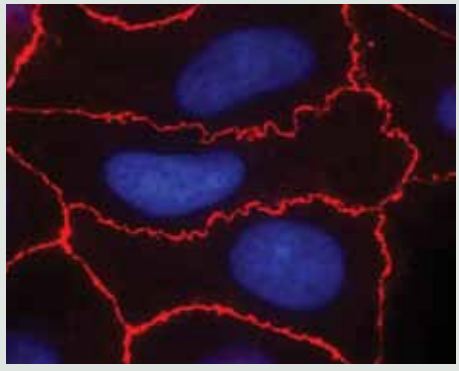

Figure 2. Visualizing tight junctions with high specificity. Human Caco-2 cells were stained with the anti–ZO-1–Alexa Fluor® 594 conjugate (Cat. No. 339194). DNA was counterstained with blue Hoechst 33258 stain (Cat. No. H3569).

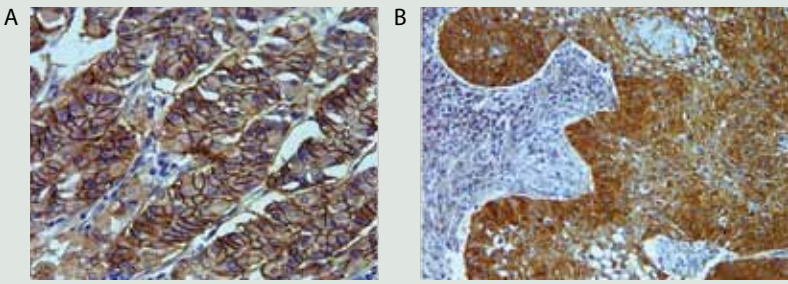

Figure 3. Normal human stomach (A) and squamous lung carcinoma (B) tissues labeled with rabbit anti–claudin-18. Formalin-fixed, paraffin-embedded (FFPE) tissues were labeled immunohistochemically with the SuperPicTure™ Kit (Cat. No. 87-8963). Image magnifications are 40x and 20x, respectively. Note membrane staining of normal stomach and cytoplasmic staining of lung carcinoma tissue.

ABfinity™ recombinant antibodies against the cell junction targets AF-6 and claudin-18 have recently been added to our extensive portfolio of antibodies. Figure 3 demonstrates immunohistochemical results using rabbit anti–claudin-18.

Tools for cell junction research

For more information about ABfinity™ technology, refer to pages 8–10 of this issue. Start exploring our extensive selection of cell junction antibodies at **www.invitrogen.com/bp61**. ■

Table 1. Alexa Fluor® dye–antibody conjugates for cell junction research.

Antigen	Conjugate	Ex (nm)	Em (nm)	Quantity	Cat. No.
Claudin-1	Alexa Fluor® 488	494	517	100 µg	374988
Claudin-4	Alexa Fluor® 488	494	517	100 µg	329488
Claudin-4	Alexa Fluor® 594	590	617	100 µg	329494
Claudin-5	Alexa Fluor® 488	494	517	100 µg	352588
Connexin 43	Alexa Fluor® 488	494	517	100 µg	138388
Occludin	Alexa Fluor® 488	494	517	100 µg	331588
Occludin	Alexa Fluor® 594	590	617	100 µg	331594
α-Tubulin	Alexa Fluor® 488	494	517	100 µg	322588
ZO-1	Alexa Fluor® 488	494	517	100 µg	339188
ZO-1	Alexa Fluor® 594	590	617	100 µg	339194

Table 2. Antibodies for cell junction research.

Product	Reactivity	Applications	Quantity	Cat. No.
AF-6, ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu, Ms (B, Cn, Cp, Mk, Rt)	WB, IHC	100 µg	700193
Cadherin-E, Mouse Monoclonal Antibody	Hu	WB, E, IP, IF, IHC, FC, Inhib	100 µg	131700
Claudin-1, Rabbit Polyclonal Antibody	Cn, Hu, Rt	WB, E, IF, IHC	100 µg	519000
Claudin-18, ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu, Ms (Cn, Cp, Eq, Mk, Rt)	WB, IHC	100 µg	700178
Connexin 43, Rabbit Polyclonal Antibody	Hu, Ms, Rt	WB, E, IHC, ICC, IF	50 µg	710700
N-Cadherin, Mouse Monoclonal Antibody	Ch, Hu, Ms, Rt, Sw	WB, IP, IF, IHC, ICC	100 µg	333900
Occludin, Mouse Monoclonal Antibody	Cn, Hu, Ms, Rt	WB, E, IF	100 µg	331500
β-III-Tubulin, Mouse Monoclonal Antibody	B, Hu, Ms, Rt	WB , IHC (FFPE samples)	100 µg	480011
ZO-1, Mouse Monoclonal Antibody	Cn, Hu	WB, E, IF	100 µg	339100

Reactivity: B = bovine; **Ch** = chicken; **Cn** = canine; **Cp** = chimpanzee; **Eq** = equine; **Hu** = human; **Mk** = monkey (rhesus); **Ms** = mouse; **Rt** = rat; **Sw** = swine. **( )** indicates reactivity predicted but not tested. **Applications: E** = ELISA; **ICC** = immunocytochemistry; **IF** = immunofluorescence; **IHC** = immunohistochemistry; **IP** = immunoprecipitation; **WB** = western blotting; **Inhib** = inhibition of E-cadherin–dependent cell-to-cell contact.

Beyond classification

CLICK-IT® EDU OUTPERFORMS BRDU ACROSS ALL SPECIES.

The Click-iT® EdU cell proliferation assay is a superior alternative to traditional methods for detecting and quantitating newly synthesized DNA. Click-iT® EdU eliminates harsh treatments required by the antibody-based BrdU method, providing an assay that is not only easier to perform but more reliable. Although the Click-iT® EdU assay has only been available since 2007, its use has already been demonstrated in a wide variety of species—spanning plants, bacteria, yeasts, and a broad spectrum of animals.

Detecting active DNA synthesis

EdU (5-ethynyl-2′-deoxyuridine) is a superior alternative to BrdU (5-bromo-2′-deoxyuridine) for directly measuring active DNA synthesis in the S phase of the cell cycle. A nucleoside analog of thymidine, EdU is incorporated into DNA during active DNA synthesis [1]. EdU detection is based on a click reaction—a copper(I)-catalyzed reaction between an azide and an alkyne. EdU contains an alkyne that reacts with an azide-containing detection reagent to form a very stable triazole ring (Figure 1).

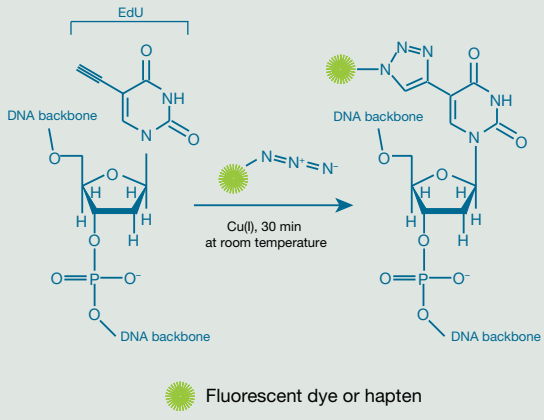

Figure 1. Click reaction between EdU and azide-modified dye or hapten.

Kingdom Eubacteria: Nondenaturing conditions give EdU the edge

The small size of the fluorescent azide (MW ~1,000) compared with an anti-BrdU antibody (MW ~150,000) enables detection of incorporated EdU using mild conditions. For BrdU detection, DNA must be denatured with DNase, heat, or HCl; these treatments can destroy antigen recognition sites or make it difficult to perform simultaneous cell cycle analysis, since many dyes for cell cycle analysis require dsDNA.

A recent publication describes the first reported use of the Click-iT® EdU assay in bacteria [2]. Ferullo and colleagues took advantage of the mild, nondenaturing conditions afforded by the assay for the study of DNA content and replication in *E. coli*. The assay enabled DNA synthesis to be visualized and quantitated while keeping cells intact for fluorescence microscopy.

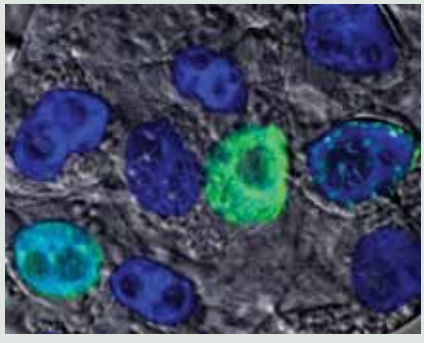

Figure 2. Cell wall digestion is not required with Click-iT® EdU. *Medicago sativa* (alfalfa) suspension cultures were incubated with 10 µM EdU for 3 hr. Cells were then fixed and permeabilized. EdU that had been incorporated into newly synthesized DNA was detected with the Click-iT® EdU Alexa Fluor® 488 Imaging Kit (green fluorescence, Cat. No. C10337). Nuclei were stained with blue-fluorescent DAPI. Six confocal sections were overlaid onto a differential interference contrast image. Image contributed by Ferhan Ayadin, Cellular Imaging Laboratory, Biological Research Center, Szeged, Hungary.

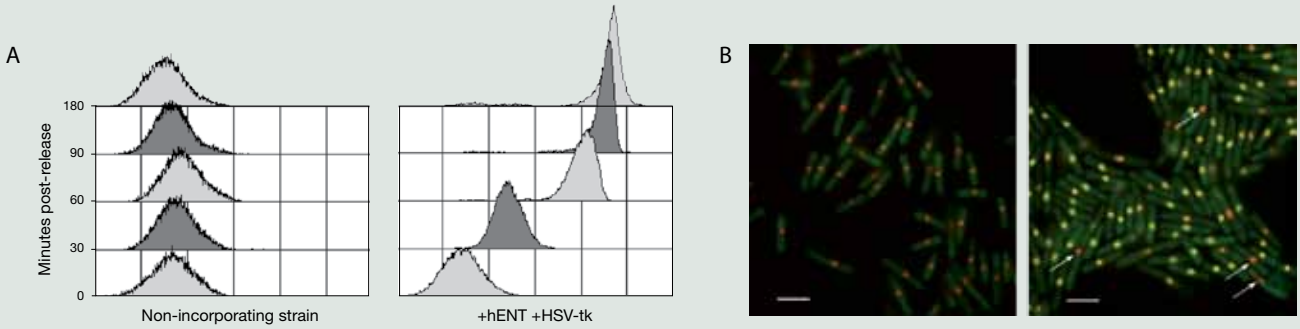

Figure 3. Assessment of DNA replication in a population of fission yeast cells. (**A**) *Schizosaccharomyces pombe* cells were arrested in S phase with hydroxyurea, then released in the presence of EdU at 32°C, and fixed in 70% ethanol at 0, 30, 60, 90, and 180 min post-release. Two wild type strains were tested for signal specificity: a non-incorporating strain (left), and a strain containing the human nucleoside transporter (hENT) plus the herpes simplex virus thymidine kinase (HSV-tk) stably integrated (right). Fixed cells were processed with the Click-iT® EdU Alexa Fluor® 488 Flow Cytometry Assay Kit (Cat. No. C35002) and run on a FACScan™ flow cytometer, 488 nm excitation (FL1, log scale). Cells capable of incorporating EdU replicate DNA and shift to the right, holding with 2C content by 90 min. Total DNA content was assessed on the same samples with SYTOX® Green (not shown), confirming that the majority of cells had completed replication and attained 2C DNA content by 90 min. (**B**) Cells were arrested in S phase with hydroxyurea, then released in the presence of EdU at 32°C. At 0 min (left) and 90 min (right) post-release, cells were fixed in 70% ethanol and processed with the Click-iT® Alexa Fluor® 488 Imaging Kit (Cat. No. C10337). Cells were fixed onto coverslips, mounted with DAPI, and imaged at 63x magnification. DNA is false-colored red, EdU signal is green. Arrows show cells that did not incorporate the EdU signal, suggesting poor release from S phase arrest or failure to arrest before completion of synthesis. Image contributed by Sarah A. Sabatinos and Susan L. Forsburg, Department of Molecular and Computational Biology, University of Southern California.

Kingdom Plantae: Cell walls are no barrier for EdU

Cell walls typically provide an additional barrier when detecting cell proliferation. Using antibodies to detect DNA synthesis not only requires DNA denaturation but also requires that the cell wall be digested. However, cell wall–digesting enzymes often contain impurities that can decrease the reliability of the assay. Even with plant cells, Click-iT® EdU assays involve only a mild fixation and permeabilization step—no DNA denaturation or cell wall digestion is required (Figure 2). Click-iT® EdU assays are faster and more accurate than antibody-based assays and are extremely sensitive and reliable, even on tough samples.

In a recent publication, cell proliferation in the *Arabidopsis* root meristem was evaluated in response to a mutation in the cell cycle switch gene *CCS52A2*. The use of the Click-iT® EdU assay enabled the role of this gene in different regions of the root to be elucidated, while keeping the root structure intact for microscopic analysis [3].

Kingdom Fungi: Perform cell cycle analysis with ease

Yeast is commonly used as a model system for studies of the cell cycle. Because yeasts lack a mechanism to transport thymidine and do not have the ability to convert thymidine to dTTP, nucleoside transporter and thymidine kinase genes must be introduced into the yeast strain of interest before performing a thymidine analog–based cell proliferation assay like the the Click-iT® EdU assay.

Figure 3 demonstrates the successful detection of nascent DNA synthesis in *Schizosaccharomyces pombe* using the Click-iT® EdU assay. A yeast strain containing the human nucleoside transporter (hENT) plus the stably integrated herpes simplex virus thymidine kinase (HSV-tk) incorporates EdU into DNA, which can be detected using either flow cytometry or fluorescence microscopy.

Kingdom Animalia: Simple EdU method wins with complex samples

The streamlined Click-iT® EdU detection protocol enables detection of cell proliferation in three easy steps:

1. Treat cells with EdU.
2. Fix and permeabilize.
3. Detect with Click-iT® detection cocktail for 30 minutes, then analyze.

The Click-iT® EdU detection protocol reduces the total number of steps and significantly decreases the total amount of time required to →

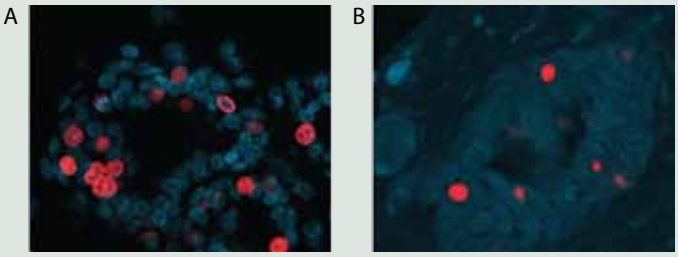

Figure 4. Stronger signal with EdU direct detection compared to BrdU secondary detection. Rats were treated with estradiol 3 days prior to a 2 hr pulse of (**A**) EdU or (**B**) BrdU administered intraperitoneally (160 µg/g body weight). Paraffin sections (5 µm) were processed, and proliferating cells, labeled red, were detected with either (**A**) a click reaction using Alexa Fluor® 594 azide supplied in the Click-iT® EdU Alexa Fluor® 594 Imaging Kit (Cat. No. C10339), or (**B**) anti-BrdU antibody followed by Alexa Fluor® 594 goat anti–mouse IgG secondary antibody (Cat. No. A21125). Nuclei were stained with blue-fluorescent Hoechst 33342 (Cat. No. H3570). In the BrdU detection method, denaturation of DNA with HCl results in a weaker nuclear signal.

perform a cell proliferation assay. This is especially beneficial for tissue samples, which often require a secondary antibody for detection of the BrdU primary antibody.

EdU direct detection also results in a stronger nuclear fluorescence signal compared to BrdU secondary detection (Figure 4). Furthermore, the non–antibody-based EdU system makes multiplexed analyses easier to design by enabling greater flexibility when selecting antibodies for other targets. The click chemistry–based EdU assay holds enormous potential for detection of DNA synthesis in a variety of animal species (Table 1, Figure 4, Figure 5).

Table 1. Use of Click-iT® EdU in animal species.

Species	Reference
Nematode (*C. elegans*)	4
Flatworm (marine)	No published reference (see Figure 5)
Cricket	5
Zebrafish larva	*BioProbes* 57*
Zebra finch	Scientific poster, ASCB 2007*
Chicken	6
Mouse	7–9
Rat	Scientific poster, ASCB 2007* (see Figure 4)
Human	10, 11

*Visit **www.invitrogen.com/bp61** to view the articles and scientific posters.

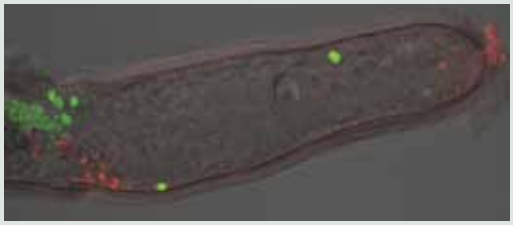

Figure 5. Detection of DNA synthesis in the flatworm *Flagellophora* cf. *apelti*. Cells were exposed to EdU (100 µM in seawater) for 10 hr. Following fixation and permeabilization, EdU that had been incorporated into newly synthesized DNA was detected with the Click-iT® EdU Alexa Fluor® 488 Imaging Kit (Cat. No. C10337, green fluorescence). Phospho-H3 was detected using a rabbit primary antibody followed by an Alexa Fluor® 568 dye–labeled goat anti–rabbit IgG antibody (Cat. No. A11011, red fluorescence). Image submitted by Julian Smith, Department of Biology, Winthrop University, USA.

Discover the benefits of Click-iT® EdU

Click-iT® EdU provides a fast, reliable, and simple cell proliferation assay for any cell type. Find out what Click-iT® EdU can do for your research at **www.invitrogen.com/bp61**. ■

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Product	Quantity	Cat. No.
Click-iT® EdU Alexa Fluor® 488 Flow Cytometry Assay	1 kit, 50 assays	C35002
Click-iT® EdU Alexa Fluor® 647 Flow Cytometry Assay		A10202
Click-iT® EdU Pacific Blue™ Flow Cytometry Assay		A10034
Click-iT® EdU Alexa Fluor® 488 Imaging Kit	1 kit, 50 coverslips	C10337
Click-iT® EdU Alexa Fluor® 555 Imaging Kit		C10338
Click-iT® EdU Alexa Fluor® 594 Imaging Kit		C10339
Click-iT® EdU Alexa Fluor® 647 Imaging Kit		C10340
EdU (5-ethynyl-2′-deoxyuridine)	50 mg	A10044
	500 mg	E10187

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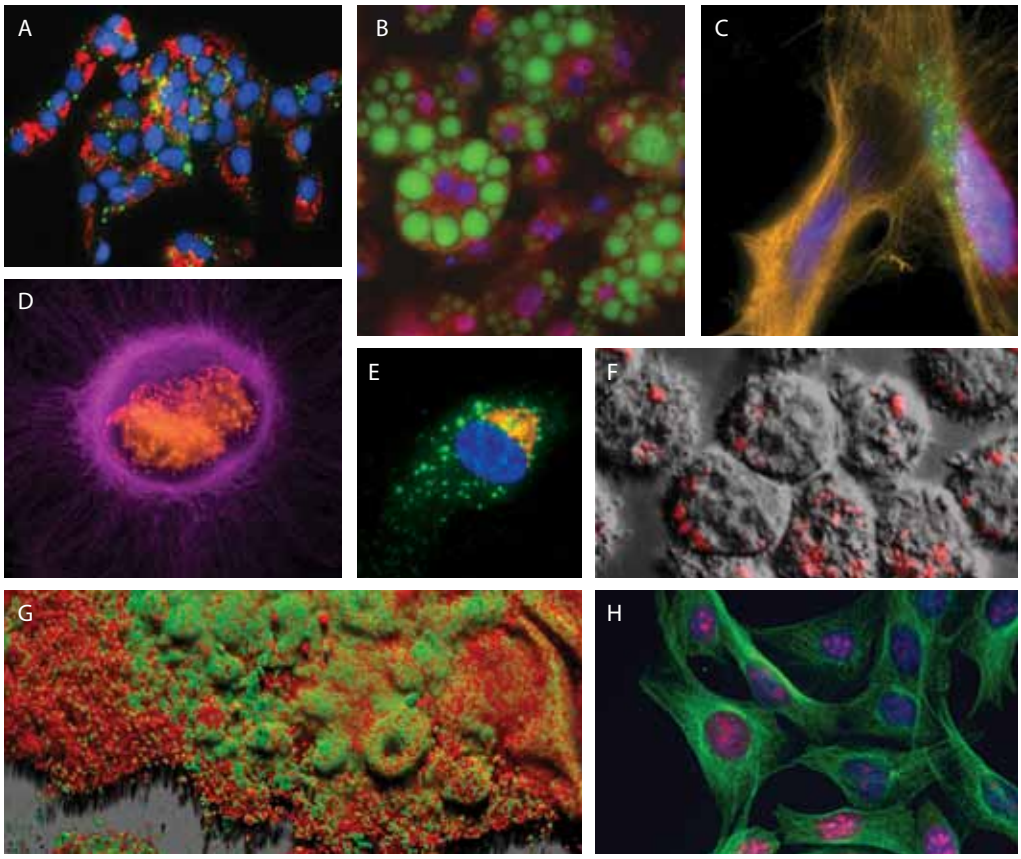

(**A**) Phospholipidosis and steatosis in HepG2 cells visualized with HCS LipidTOX™ Red Phospholipidosis Detection Reagent (red, Cat. No. H34351), HCS LipidTOX™ Green Neutral Lipid Stain (green, Cat. No. H34475), and Hoechst 33342 (blue, Cat. No. H3570).

(**B**) Adipogenesis detected with anti–fatty acid binding protein (FABP4) antibody and visualized with Alexa Fluor® 594 goat anti–rabbit IgG antibody (red, Cat. No. A11037), LipidTOX™ Green Neutral Lipid Stain (green, Cat. No. H34475), DAPI (blue, Cat. No. D1306), and ProLong® Gold Antifade Reagent (Cat. No. P36930).

(**C**) Late-stage apoptosis imaged with Click-iT® TUNEL Alexa Fluor® 647 Imaging Assay (Cat. No. C10247), anti–caspase-3 antibody visualized with Alexa Fluor® 488 goat anti–rabbit IgG (green, Cat. No. A11008), anti-tubulin antibody visualized with Alexa Fluor® 555 goat anti–mouse IgG (orange, Cat. No. A21422), and Hoechst 33342 (blue, Cat. No. H3570).

(**D**) Proliferation of HeLa cell detected with Click-iT® EdU 555 Imaging Kit (orange, Cat. No. C10338). Tubulin was detected with an anti-tubulin antibody and visualized with Alexa Fluor® 647 goat anti-mouse IgG (purple, Cat. No. A21235).

(**E**) Vesicle trafficking in human umbilical vein endothelial cell (Cat. No. C0035C) visualized with Organelle Lights™ Endosomes-GFP (Cat. No. O10104), Organelle Lights™ Golgi-OFP (Cat. No. O36224), and Hoechst 33342 (Cat. No. H3570).

(**F**) Internalization and acidification during phagocytosis in Murine J774A.1 cells detected with pHrodo™ *E. coli* BioParticles® conjugate (Cat. No. P35361). Image contributed by Lucy Deriy and Deborah Nelson, University of Chicago.

(**G**) Viability analysis of a *Staphylococcus epidermidis* biofilm using the FilmTracer™ LIVE/DEAD® Biofilm Viability Kit (Cat. No. L10316). Image contributed by Betsey Pitts, Montana State University.

(**H**) RNA synthesis in NIH/3T3 cells detected with Click-iT® RNA Alexa Fluor® 594 Imaging Kit (Cat. No. C10330). Tubulin was detected with mouse anti-tubulin IgG and visualized with Alexa Fluor® 488 goat anti–mouse IgG (green, Cat. No. A10229), and nuclei were stained with Hoechst 33342 (Cat. No. H3570).

Fluorescence-based alternatives to chromogenic immunohistochemistry

QUANTITATIVE, MULTIPLEXED RESULTS WITH FLUORESCENT DYES, REFERENCE BEADS, AND ANTIFADE REAGENTS.

Immunohistochemistry (IHC) exploits the binding of antibodies to target proteins to detect their localization in cells and tissues. IHC protocols, especially in pathology, are dominated by methods based on enzyme-coupled secondary antibodies that are detected using chromogenic substrates. Yet standard IHC techniques are limited to semiquantitative analysis, are often limited to one or two analytes, and because enzymatic reactions tend to saturate, have a narrow dynamic range. This is a serious complication for cancer diagnostics as the number of biomarkers increases, demanding multiplexed results with improved quantitation. In perhaps the most dramatic case illustrating the limitations of current IHC methodology, 400 out of 1,000 breast cancer pathology slides were misdiagnosed over an 8-year period, estimated to have led to the deaths of 100 patients. Change in this vast industry is slow to occur, and many of the challenges have been well outlined [1].

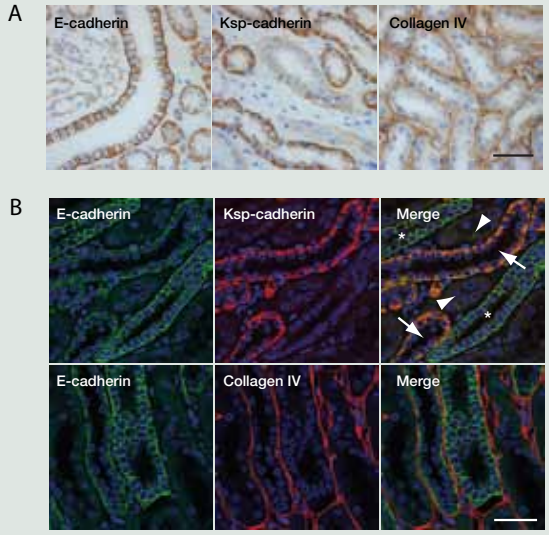

Figure 1. Greater dynamic range and multiplexing capabilities of fluorescence-based IHC. FFPE sections (3 µm) of adult human kidney were dewaxed, rehydrated, and heated to 95°C for 30 min. Sections were stained with anti–E-cadherin and anti–Ksp-cadherin antibodies or with anti–E-cadherin and anti–collagen IV antibodies, followed by (**A**) HRP-based secondary conjugates, or (**B**) Alexa Fluor® 488–conjugated goat anti–mouse IgG2a (Cat. No. A21131) and Alexa Fluor® 555 goat anti–mouse IgG1 (Cat. No. A21127), or Alexa Fluor® 488–conjugated goat anti–mouse IgG2A and Alexa Fluor® 555 goat anti–rabbit Ig (Cat. No. A21428). Nuclei were counterstained with DAPI (Cat. No. D1306). Arrows indicate tubules expressing both cadherins; arrowheads indicate tubules expressing neither cadherin. *Indicates tubules expressing E-cadherin alone. Scale bar = 50 µm. Reproduced from BioMed Central, *BMC Cell Biol* 9:13 (2008), courtesy of D Robertson and CM Isacke.

Advantages of fluorescence-based approaches

Many recent publications [2–10] show convincingly that protocols incorporating either fluorescent organic dyes or inorganic Qdot® nanocrystals, along with superior antifade mountants and intensity calibration standards, can replace traditional chromogenic IHC methods, with improved results. Fluorescence-based methods provide many of the advantages of traditional approaches, including ease of use and excellent, stable signal amplification. They also benefit from broader dynamic ranges and the improved resolving power of direct conjugates compared to enzyme conjugates. These fluorescent labels also demonstrate an extended storage life and offer the possibility of adding 2–5 or more separate labels in a single experiment (Figures 1 and 2). Several companies, such as Aureon and HistoRx, are already commercializing fluorescence-based pathology services.

Bringing accurate quantitation to immunohistochemistry

Variation in samples, equipment, and reagents presents a significant challenge for quantifying fluorescence intensities and, by inference, protein levels. Invitrogen's InSpeck™ fluorescent microspheres overcome this obstacle by providing intensity references to correct against experimental fluctuations. Recent publications [3,4] advocate that, in the same way that flow cytometry good practices incorporate calibration beads routinely, similar practices be implemented to bring accurate quantitation

to IHC. These protocols begin by determining the intensities of 200 to 400 individual 6 µm InSpeck™ beads. To expand the dynamic range of fluorescence intensity, images are captured using12-bit grayscale with a black-and-white CCD, yielding an expansion of the colorimetric color scale from 0–4 to 0–4,094 units. The mean pixel intensities are then used to normalize intensities from the previous imaging session. Invitrogen offers these reference beads as stand-alone solutions of single intensities, or mounted on microslides in optical cement as a complete intensity series.

Preserving signal intensity

These same studies demonstrate the ability of ProLong® Gold Antifade Reagent to preserve fluorescence signal intensity for up to 2 months at –20°C. More importantly, there was no detectable decline in signal intensity (fading or bleaching) during image capture sessions. Other laboratories have examined the stability of Alexa Fluor® dyes in longer-term studies [2], in which a stable signal was observed for over 9 months (Figure 2).

Alternatively, Qdot® nanocrystals are being considered extensively for quantitative fluorescence intensity analysis with IHC samples [10]. The brightness, photostability, and narrow emission profiles of Qdot® nanocrystals enable direct antibody labeling, with the potential to substantially improve IHC workflows. Indeed, nearly 100 citations demonstrate the use of nanocrystals for IHC, showing that the concept has merit (for example, see references 5, 7, and 8). Five-color multiplex results have been obtained, and by using PBS washes and aqueous mounting media, extended shelf life of over 18 months has been demonstrated. For optimal signal preservation and image quality when using Qdot® nanocrystals, we recommend using our new Qmount™ Qdot® Mounting Media.

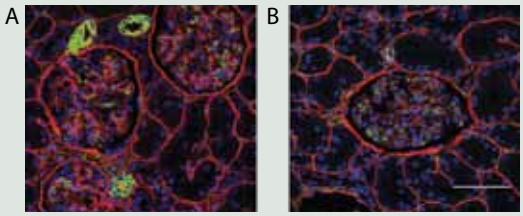

Figure 2. Stability of Alexa Fluor® dye fluorescence for up to 270 days in immunohistochemical sections. Sections of human kidney were triple stained as described in Figure 1, first with rabbit anti–collagen IV antibody detected with Alexa Fluor® 555 goat anti–rabbit IgG secondary antibody (red, Cat. No. A21429), and then with mouse anti–alpha smooth muscle actin antibody detected with Alexa Fluor® 488–conjugated goat anti-mouse IgG2a (green, Cat. No. A21131). Nuclei were counterstained with DAPI (blue, Cat. No. D1306). Sections were imaged on (**A**) day 1 and (**B**) after storage for 270 days at –20°C. No appreciable decline in imaging intensity was observed. Scale bar = 50 µm. Reproduced from BioMed Central, *BMC Cell Biol* 9:13 (2008), courtesy of D Robertson and CM Isacke.

The future of immunohistochemistry

Standard colorimetric assays continue to dominate IHC methodology, and we offer many kits and assays based on this approach. However, the improved resolution and quantitation capabilities of fluorescence-based approaches show promise for the future of immunohistochemistry. Explore our antibody and Qdot® nanocrystal offerings for IHC at www.invitrogen.com/bp61. ■

References

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4. Huang D, Casale GP, Tian J et al. (2009) *Int J Cancer* Aug 12 [Epub ahead of print].
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9. Tholouli F, Sweeney E, Barrow E (2008) *J Pathol* 216:275–285.
10. Medintz IL, Mattoussi H, Clapp AR (2008) *Int J Nanomedicine* 3:151–167.

Product	Quantity	Cat. No.
Antifade and mounting reagents		
ProLong® Gold Antifade Reagent, special packaging	5 x 2 mL	P36934
ProLong® Gold Antifade Reagent	10 mL	P36930
ProLong® Gold Antifade Reagent with DAPI, special packaging	5 x 2 mL	P36935
ProLong® Gold Antifade Reagent with DAPI	10 mL	P36931
Qmount™ Qdot® Mounting Media	3 x 2 mL	Q10336
Stand-alone bead solutions		
InSpeck™ Green (505/515) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit	I7219
InSpeck™ Blue (350/440) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit	I7221
InSpeck™ Orange (540/560) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit	I7223
InSpeck™ Red (580/605) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit	I7224
InSpeck™ Deep Red (633/660) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit	I7225
InSpeck™ Green (505/515) Microscope Image Intensity Calibration Kit, 6 µm	1 kit	I14785
InSpeck™ Orange (540/560) Microscope Image Intensity Calibration Kit, 6 µm	1 kit	I14786
InSpeck™ Red (580/605) Microscope Image Intensity Calibration Kit, 6 µm	1 kit	I14787
Prepared slides		
FocalCheck™ Fluorescence Microscope Test Slide #1, for alignment, intensity, and calibration	1 each	F36909
FocalCheck™ Fluorescence Microscope Test Slide #3, 5 colors, high and low intensities	1 each	F36914

Accurate compensation is as easy as AbC

ABC™ AND ARC™ KITS FOR FLOW CYTOMETRY COMPENSATION.

Multicolor flow cytometry is a powerful technique, offering the ability to study and quantify several cell biology parameters simultaneously. The accuracy of this technique, however, depends on the researcher's ability to assign the various fluorescence intensity measurements to the correct fluorochromes. Often, the fluorescence emission profile from one fluorochrome overlaps those of other nearby fluorochromes. Removing this spectral overlap can be difficult, especially in situations where the antigen of interest is expressed at a low level or the researcher is trying to consume as little sample as possible to obtain the compensation data.

To respond to this need for more accurate compensation tools, we have developed kits that work with dye-labeled antibodies: the AbC™ Bead Kits (for mouse, rat, or hamster antibodies); and a kit that corrects spectral overlap when using our LIVE/DEAD® amine-reactive dyes: the ArC™ Reactive Bead Compensation Kit. These kits are supplied with polystyrene beads that have the approximate autofluorescence and size of human lymphocytes. Polystyrene beads are ideal for setting compensation because they provide consistent signals with bright intensities every time they are stained. Additionally, these products are useful when cell antigen expression is dim or absent and when cell number is limited, to preserve cells for panel testing.

Antibody capture compensation kits

The AbC™ Anti-Mouse Bead Kit and the AbC™ Anti-Rat/Hamster Bead Kit provide sensitive and simple-to-use tools for the setting of flow cytometry compensation in experiments employing fluorochrome-conjugated mouse, rat, or hamster antibodies. Each kit contains two types of modified polystyrene microspheres: the AbC™ capture beads for binding immunoglobulin, and the negative beads (possessing no antibody-binding capacity). The kits provide superior and consistent binding of key antibody isotypes. The AbC™ Anti-Mouse Bead Kit capture beads react with all mouse antibody isotypes, including IgG1, IgG2a, IgG2b, IgG3, and IgM. The AbC™ Anti-Rat/Hamster Bead Kit capture beads react with rat isotypes IgG1, IgG2a, IgG2b, IgM, and hamster IgG.

After incubation with a fluorochrome-conjugated antibody, the bead components provide distinct positive and negative populations that can be used to set compensation, either manually or using

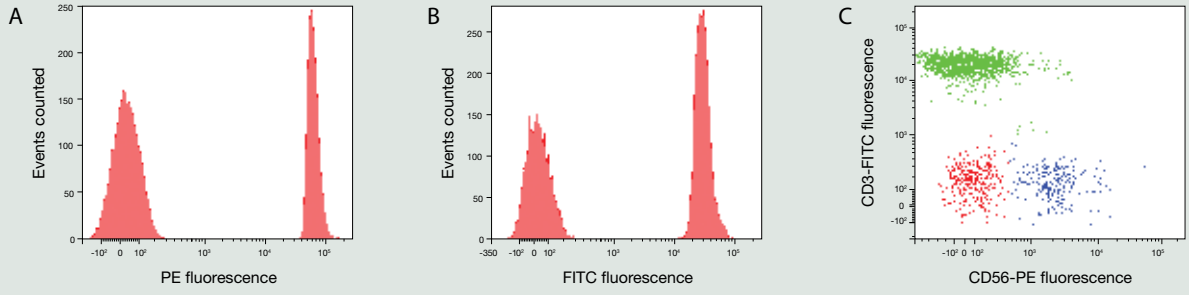


Figure 1. Compensation using the AbC™ Anti-Mouse Bead Kit. (**A**) Phycoerythrin (PE)-conjugated mouse anti–human CD56 antibody (Cat. No. MHCD56044) was used to label AbC™ capture beads, providing a positive signal; AbC™ negative beads provide the negative signal. (**B**) FITC-conjugated mouse anti–human CD3 (Cat. No. MDCH03014) was used to label AbC™ capture beads, providing a positive signal; AbC™ negative beads provide the negative signal. (**C**) Dual-parameter plot showing gated human lymphocytes labeled with both PE-conjugated mouse anti–human CD56 and FITC-conjugated mouse anti–human CD3 using compensation settings obtained with the AbC™ Anti-Mouse Bead Kit.

instrument software. This ensures that compensation is performed with the same fluorochrome-labeled antibody that is used for cell staining. The consistent nature of polystyrene beads and high antibody-binding capacity provides more consistent and accurate compensation for all combinations of fluorochrome-labeled antibodies (Figure 1).

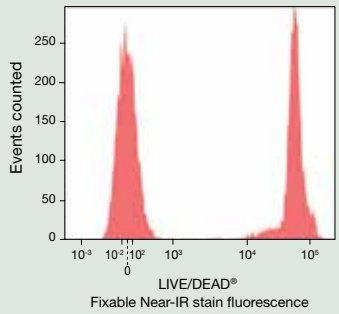


Figure 2. Staining profile of the ArC™ Kit components with the LIVE/DEAD® Fixable Near-IR Dead Cell Stain Kit. ArC™ reactive beads were stained with LIVE/DEAD® fixable near-IR dye, washed, mixed with the negative beads, and analyzed using 633 nm excitation with emission collected using a 780/60 nm bandpass filter.

Compensation with LIVE/DEAD® fixable dead cell stains

Excluding dead cells in immunophenotyping and functional assays is an important step for accurate flow cytometric analysis. The ArC™ Amine Reactive Compensation Bead Kit is designed to facilitate compensation when using any of the eight amine-reactive dyes of our LIVE/DEAD® fixable dead cell stains. Each of these kits provides two types of modified polystyrene microspheres: the ArC™ reactive beads that can be labeled with any of the amine-reactive dyes, and the ArC™ negative beads (possessing no amine reactivity). We have optimized the ArC™ kit protocol for each of the eight LIVE/DEAD® fixable stains to ensure that you get maximum performance regardless of which one you use. Using the two kit components with any amine-reactive dye will provide distinct positive and negative populations that can be used to set compensation, either manually or using instrument software (Figure 2).

Combining AbC™ and ArC™ compensation kits

The AbC™ Bead Kits can be used together with the ArC™ Amine Reactive Compensation Bead Kit to calculate compensation in multicolor immunophenotyping experiments that incorporate a LIVE/DEAD® fixable dye, providing a complete solution for compensation controls. Detailed procedures for combining the kits for setting compensation in one experiment are included in the product insert instructions.

Get the simpler solution to flow cytometry compensation

To find out more about the AbC™ and ArC™ kits for accurate flow cytometry compensation, visit **www.invitrogen.com/bp61**. ■

Product	Quantity	Cat. No.
AbC™ Anti-Mouse Bead Kit, for mouse antibody capture for flow cytometry compensation	100 tests	A10344
AbC™ Anti-Rat/Hamster Bead Kit, for rat/hamster antibody capture for flow cytometry compensation	100 tests	A10389
ArC™ Amine Reactive Compensation Bead Kit, for use with amine-reactive dyes for flow cytometry compensation	100 tests	A10346
LIVE/DEAD® Fixable Near-IR Dead Cell Stain Kit, for 633 or 635 nm excitation	200 assays	L10119
LIVE/DEAD® Fixable Far Red Dead Cell Stain Kit, for 633 or 635 nm excitation	200 assays	L10120
LIVE/DEAD® Fixable Green Dead Cell Stain Kit, for 488 nm excitation	200 assays	L23101
LIVE/DEAD® Fixable Red Dead Cell Stain Kit, for 488 nm excitation	200 assays	L23102
LIVE/DEAD® Fixable Blue Dead Cell Stain Kit, for UV excitation	200 assays	L23105
LIVE/DEAD® Fixable Violet Dead Cell Stain Kit, for 405 nm excitation	200 assays	L34955
LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit, for 405 nm excitation	200 assays	L34957
LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit, for 405 nm excitation	200 assays	L34959
LIVE/DEAD® Fixable Dead Cell Stain Sampler Kit for flow cytometry	320 assays	L34960

Measuring insulin receptors in cell lysates

QUANTIFY SITE–SPECIFIC PHOSPHOPROTEINS USING PHOSPHOELISA™ KITS.

Insulin is secreted by beta cells of the islets of Langerhans in the pancreas and plays a key role in regulating plasma glucose levels. Binding of insulin and insulin-like growth factor 1 (IGF-1) to the insulin receptor (IR) and IGF-1 receptor (IGF-1R) results in a cascade of signaling events, beginning with the autophosphorylation of the receptor and the activation of intrinsic tyrosine kinase activity, which allows appropriate substrates to be phosphorylated. IGF-1 has 48% amino acid sequence identity with proinsulin and is capable of binding to IR, but with less affinity than insulin. Functionally, the stimulation or inhibition of these pathways can affect metabolic regulation, growth, and differentiation.

Measuring total and phosphorylated receptors

We have developed phosphoELISA™ kits to measure levels of total and phosphorylated IR and IGF-1R proteins. The phosphoELISA™ method is a simple, fast, and unbiased way to quantify protein levels with high specificity and sensitivity in cell lysates. The kits come ready to use with all necessary reagents, including recombinant standards to be used as positive controls and to quantitate results.

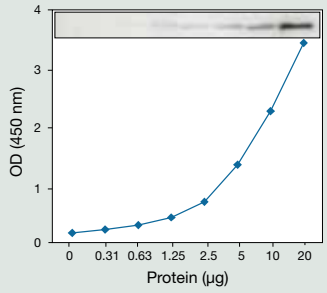


Figure 1. Superior sensitivity of ELISA compared to western blotting to detect IR [pYpY1162/1163]. As the amount of protein decreases, western blot bands become difficult to distinguish. Bands shown in the western blot were detected using rabbit anti-IR [pYpY1162/1163] and an alkaline phosphatase–conjugated anti-rabbit IgG, followed by chemiluminescent substrate addition and autoradiography. The IR [pYpY1162/1163] phosphoELISA™ kit (Cat. No. KHR9131) was used to generate the quantitative results.

Superior specificity, quantitative results

Why use phosphoELISA™ kits to augment your research? The enzyme-linked immunosorbent assay (ELISA) provides high specificity due to the use of two antibodies to detect the antigen of interest (sandwich antibody pair), decreasing the chances of nonspecific binding. In addition, with an ELISA readout, extrapolation from the standard curve allows for quantitative results, which eliminates the need for densitometry analysis of western blots.

ELISAs also provide a 2x to 10x sensitivity increase over western blots, which means less cell lysate is needed to run your experiments and low-expression proteins can be measured. By combining western blot images of specific protein bands with optical density data from phosphoELISA™ assays, you get a complete qualitative and quantitative story (Figure 1).

Each phosphoELISA™ kit is phosphospecific

Specificity testing for each phosphoELISA™ kit is performed by peptide competition studies. For example, to test specificity of the IR [pYpY1162/1163] ELISA kit, the assay is run as usual, with the exception that the detection antibody is preincubated with IR-derived peptides at varying concentrations (Figure 2). The expected result is that of all the IR-derived peptides incubated, only the peptide containing phosphorylated tyrosines 1162/1163 is able to block the ELISA signal. IR-derived peptides phosphorylated at other sites do not block the signal, even at high concentrations.

Specificity testing is also performed on well-established cell model systems with known stimulating or inhibiting effects (Figure 3).

Normalization of phosphorylated protein measurement to total protein measurement is important to verify whether a particular treatment affects the amount of total protein as well as specific phosphorylation. As shown in Figure 3, phosphorylation of IGF-1R is upregulated upon IGF-1 treatment of MCF-7 cells. Total protein levels of IGF-1R remain unchanged after treatment with IGF-1 protein, demonstrating the specific increase in IGF-1R phosphorylation.

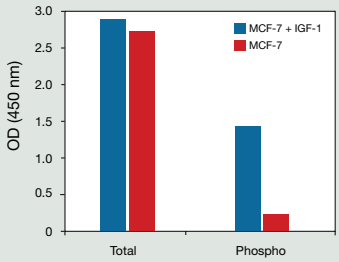


Figure 3. Increased IGF-1R phosphorylation following IGF-1 treatment. MCF-7 cells were pretreated with 1 mM sodium orthovanadate for 16 hr, then treated with IGF-1 at 100 ng/mL for 15 min. Untreated MCF-7 cells were used as a control. Cell extracts were prepared, and cell lysates (400 mg/mL) were analyzed with the IGF-1R [pYpY1135/1136] ELISA Kit (Cat. No. KHO0501) and the IGF-1R (β-subunit) ELISA Kit (Cat. No. KHO0491).

Your source for metabolic signaling research

We also offer the Insulin ELISA Kit for insulin measurement in human serum or plasma, IGF-1 recombinant protein for treatment of cells, phospho-site–specific antibodies such as IR/IGF-1R [pYpY1162/1163] for western blotting, and multiplexing assays for the Luminex® platform. For more information on metabolic signaling pathway products, visit www.invitrogen.com/bp61. ■

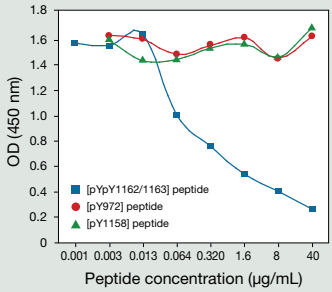
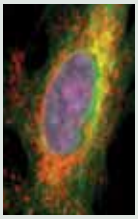


Figure 2. Peptide blocking studies show specificity of the IR [pYpY1162/1163] ELISA kit. Peptides in a range of concentrations were incubated with the detector antibody. Only the peptide corresponding to the region surrounding tyrosines 1162/1163, containing the phosphotyrosines, was able to block the ELISA signal. The peptides containing phosphorylated tyrosine at positions 972 and 1158 did not block the signal.

Product	Quantity	Cat. No.
ELISA kits		
Insulin Human ELISA Kit	96 tests	KAQ1251
IR (β-subunit) ELISA Kit	96 tests	KHR9111
IR [pYpY1162/1163] ELISA Kit	96 tests	KHR9131
IR [pY1158] ELISA Kit	96 tests	KHR9121
IGF-1R (β-subunit) ELISA Kit	96 tests	KHO0491
IGF-1R [pYpY1135/1136] ELISA Kit	96 tests	KHO0501
Recombinant proteins		
Recombinant Human IGF-1	10 µg	PHG9074
	25 µg	PHG9075
	100 µg	PHG9071
	1 mg	PHG9073
Antibodies		
IR/IGF-1R [pYpY1162/1163]	100 µg	700393
Luminex® assays		
Akt Phospho 7-plex Panel Akt [pS473], GSK-3β [pS9], IGF-1R [pYpY1135/1136], IR [pY1162/1163], IRS-1 [pS312], p70S6K [pTpS421/424], PRAS40 [pT246]	100 tests	LHO0001
Akt Total 7-plex Panel Akt, GSK-3β, IGF-1R, IR, IRS-1, p70S6K, PRAS40	100 tests	LHO0002

QMOUNT™ QDOT® MOUNTING MEDIA

Qmount™ Qdot® Mounting Media is a nonaqueous, permanent mountant designed for samples labeled with Qdot® nanocrystals. Unlike other commercially available mountants, Qmount™ media causes no significant loss of Qdot® fluorescence, both initially and over the course of several months. Qmount™ Qdot® Mounting Media hardens within 12 hours and has a refractive index of 1.5 when fully cured. The ready-to-use Qmount™ media is supplied in 3 convenient dropper bottles, each containing sufficient material to mount up to 20 coverslips. Learn more about Qmount™ Qdot® Mounting Media at **www.invitrogen.com/bp61**.

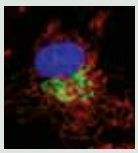


Human carcinoma (HeLa) cell labeled with Qdot® nanocrystals and mounted with Qmount™ media. Mitochondria were detected with anti–OxPhos Complex V inhibitor protein IgG (Cat. No. A21355) and labeled with Qdot® 625 goat F(ab')$_2$ anti–mouse IgG (Cat. No. A10195, red fluorescence); the Golgi apparatus was detected with rabbit anti-giantin and labeled with Qdot® 585 goat F(ab')$_2$ anti–rabbit IgG conjugate (Cat. No. Q11411MP, yellow fluorescence); tubulin was detected with rat anti-tubulin and labeled with DSB-X™ biotin goat anti–rat IgG (Cat. No. D20697) and Qdot® 525 streptavidin conjugate (Cat. No. Q10141MP, green fluorescence). The nucleus was labeled with Qnuclear™ Deep Red Stain (Cat. No. Q10363, purple fluorescence), and the slide was mounted with Qmount™ Qdot® Mounting Media (Cat. No. Q10336).

Product	Quantity	Cat. No.
Qmount™ Qdot® Mounting Media	3 x 2 mL	Q10336

BACMAM ENHANCER KIT

The BacMam Enhancer Kit provides researchers using BacMam reagents—including Premo™ biosensors for chloride, sodium, and cell cycle, and Organelle Lights™ and Cellular Lights™ reagents—with additional BacMam enhancer. BacMam enhancer helps to increase expression of BacMam reagents in mammalian cells. Different cell types vary in their response to the BacMam enhancer, and for best results the amount and treatment time should be optimized. Each kit provides sufficient materials for treating 100 coverslips or ten 96-well microplates. Learn more about BacMam technology–based products at **www.invitrogen.com/bp61**.

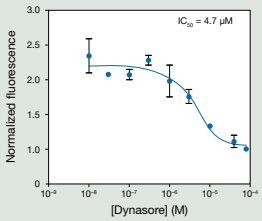


Live-cell visualization of the Golgi complex and mitochondrial dynamics and organization using BacMam technology. HeLa cells were incubated with Organelle Lights™ Golgi-GFP (Cat. No. O36215) and Organelle Lights™ Mito-RFP (Cat. No. O36229), followed by treatment with BacMam enhancer. Nuclei were stained with Hoechst 33342 (Cat. No. 3570). Cells were washed and incubated overnight to allow protein expression. Imaging was performed on live cells using a DeltaVision® Core microscope and standard DAPI/FITC/TRITC filter sets.

Product	Quantity	Cat. No.
BacMam Enhancer Kit	1 kit	B10107

PHRODO™ DEXTRAN

pHrodo™ dextran is a superior alternative to fluorescein- and rhodamine-based dextran conjugates for live-cell imaging of endocytosis. pHrodo™ dextran has a pH-sensitive fluorescence emission that increases in intensity with increasing acidity. Upon internalization of pHrodo™ dextran, the acidic environment of the endosomes elicits a bright red–fluorescent signal that can be visualized by fluorescence microscopy, flow cytometry, or high-content imaging. The minimal fluorescent signal from the pHrodo™ dextran conjugate at neutral pH prevents the detection of noninternalized and nonspecifically bound conjugates, and eliminates the need for quenching reagents and extra wash steps, thus providing a simple fluorescence assay for endocytotic activity. pHrodo™ dextran has excitation/emission maxima of ~560/585 nm, which facilitates multiplexing with other fluorophores including blue-, green-, and far-red–fluorescent probes. Localization of pHrodo™ dextran can be established using Organelle Lights™ Endosomes-GFP or Organelle Lights™ Lysosomes-GFP reagents. Learn more at **www.invitrogen.com/bp61**.



Tracking endocytosis inhibition with pHrodo™ dextran. HeLa cells were plated in 96-well format and treated with dynasore. Cells were then incubated with pHrodo™ dextran (Cat. No. P10361) and stained with HCS NuclearMask™ Blue Stain (Cat. No. H10325) to reveal total cell number and demarcation for imaging analysis. Images were acquired using the BD Pathway™ 855 High-Content Bioimager (BD Biosciences).

Product	Quantity	Cat. No.
pHrodo™ dextran, 10,000 MW, for endocytosis	0.5 mg	P10361

NEW FEATURES AND SERVICES FOR THE COUNTESS™ INSTRUMENT

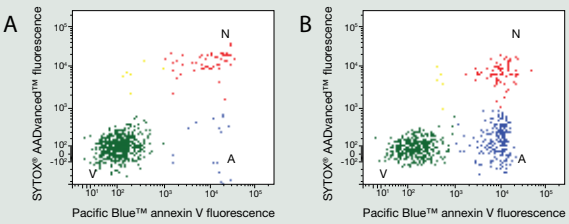
The Countess™ Automated Cell Counter offers new features that provide even greater accuracy and cell-counting control both before and after data are generated. The instrument now provides viability validation, cell gating, and a personal protocol maker. In addition, you can now purchase an extended warranty to add coverage to your existing one-year manufacturer's warranty.* Learn more about the new features and services at **www.invitrogen.com/bp61**.

Product	Quantity	Cat. No.
Countess™ Automated Cell Counter	1 each	C10227
Countess™ Automated Cell Counter Starter Kit *(includes 1 cell counter and 11 boxes of slides)*	1 kit	C10310

*The extended warranty is available in North America only.

PACIFIC BLUE™ ANNEXIN V/SYTOX® AADVANCED™ APOPTOSIS KIT

The Pacific Blue™ Annexin V/SYTOX® AADvanced™ Apoptosis Kit provides a rapid, convenient flow cytometry assay for accurate detection of early- and late-apoptotic cells. The kit contains recombinant annexin V conjugated to the violet-excitable Pacific Blue™ dye to identify apoptotic cells, and red-fluorescent SYTOX® AADvanced™ Dead Cell Stain to identify necrotic cells based on membrane integrity. After staining, apoptotic cells show bright violet fluorescence, dead cells show red fluorescence, and live cells show dim violet fluorescence. Because there is very little spectral overlap between the two dyes, very little or no compensation is required. By moving annexin V to the less commonly used violet laser, you can maximize instrument capability and measure additional apoptotic parameters. Learn more at **www.invitrogen.com/bp61**.

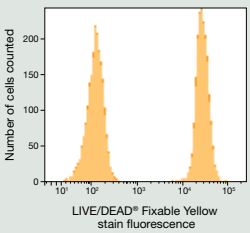


Detecting apoptosis with the Pacific Blue™ Annexin V/SYTOX® AADvanced™ Apoptosis Kit. Jurkat cells (human T cell leukemia) were (**A**) untreated or (**B**) treated with camptothecin. Cells were treated with the reagents in the Pacific Blue™ Annexin V/SYTOX® AADvanced™ Apoptosis Kit and analyzed by flow cytometry using 405 nm and 488 nm excitation. Note that the camptothecin-treated cells have a higher percentage of apoptotic cells than the basal level of apoptosis seen in the control cells. A = apoptotic cells, V = viable cells, N = necrotic cells.

Product	Quantity	Cat. No.
Pacific Blue™ Annexin V/SYTOX® AADvanced™ Apoptosis Kit	50 assays	A35136

LIVE/DEAD® FIXABLE YELLOW DEAD CELL STAIN KIT

The LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit enables you to differentially stain live and dead cells in a variety of mammalian cell types. Cells can then be fixed for subsequent analysis by flow cytometry. The kit is based on the reaction of a fluorescent reactive dye—LIVE/DEAD® Fixable Yellow dye—with amines on cellular proteins. In viable cells, only cell surface proteins are available to react with the dye, resulting in faint staining. In contrast, the dye can penetrate the interior of dead cells, resulting in at least a 50-fold increase in fluorescence compared to live cells. Because the dye reacts covalently with proteins, the staining pattern is completely preserved following formaldehyde fixation under conditions that inactivate pathogens. The spectral properties of LIVE/DEAD® Fixable Yellow dye (excitation/emission ~405/570 nm) allow you to transfer your dead-cell discrimination assays to the violet laser, freeing the commonly used 488 nm laser and making the kit ideal for multicolor experiments. Learn more at **www.invitrogen.com/bp61**.



LIVE/DEAD® Fixable Yellow dead-cell staining in Jurkat cells. The LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit (Cat. No. L34959) was used to differentially stain a mixture of live (left peak) and heat-treated (right peak) Jurkat cells. Following the staining reaction, cells were fixed in formaldehyde and analyzed by flow cytometry using 405 nm excitation and ~575 nm emission.

Product	Quantity	Cat. No.
LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit	1 kit	L34959

CLICK-IT® EDU ALEXA FLUOR® 555 ASSAYS

The Click-iT® EdU assay is a superior alternative to other assays that detect cell proliferation via nascent DNA synthesis through the incorporation of a modified nucleoside—specifically, ^{3}H-thymidine or bromodeoxyuridine (BrdU). The Click-iT® EdU assay does not use radioactivity and, in contrast to BrdU assays, eliminates the need for DNA denaturation steps. This maintains sample morphology, antigen-recognition sites, and dsDNA integrity. Furthermore, the streamlined Click-iT® EdU protocol enables detection of cell proliferation in just three easy steps. The Click-iT® EdU assay is now available with Alexa Fluor® 555 dye for detection using fluorescence microscopy or high-content imaging and analysis. Alexa Fluor® 555 dye is significantly more photostable and produces brighter conjugates than either tetramethylrhodamine (TRITC) or Cy®3 dye. This dye also enables multiplexed analyses with blue-, green-, and far-red–fluorescent dyes using standard TRITC filter sets. Learn more at **www.invitrogen.com/bp61**.

Cell proliferation visualized using the Click-iT® EdU Alexa Fluor® 555 assay. HeLa cells were treated with EdU. Cells were fixed and permeabilized, and EdU that had been incorporated into nascent DNA was detected with red-orange Alexa Fluor® 555 azide from the Click-iT® EdU Alexa Fluor® 555 Imaging Kit (Cat. No. C10338). Tubulin was labeled with a mouse anti-tubulin antibody and visualized with far-red–fluorescent Alexa Fluor® 647 goat anti-mouse IgG (Cat. No. A21235).

Product	Quantity	Cat. No.
Click-iT® EdU Alexa Fluor® 555 Imaging Kit, for 50 coverslips	1 kit	C10338
Click-iT® EdU Alexa Fluor® 555 HCS Assay, 2-plate size	1 kit	C10352
Click-iT® EdU Alexa Fluor® 555 HCS Assay, 10-plate size	1 kit	C10353

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Recently published

A LOOK AT HOW YOUR FELLOW RESEARCHERS ARE USING INVITROGEN™ PRODUCTS.

Histopathology: An optimized method for high-resolution immunofluorescence imaging of FFPE tissue samples. Pathology labs typically employ complementary methods to detect target proteins in tissue specimens: immunohistochemical (IHC) analysis and immunofluorescence imaging. However, each method has its limitations; immunofluorescence offers better resolution and a more quantitative approach, but the inherent sample autofluorescence of FFPE sections limits its use to cryosection samples that provide less morphological detail. Researchers at the Institute of Cancer Research in London have optimized a method allowing high-resolution immunofluorescence analysis of FFPE samples. Keys to the success of their method include the use of thin (hence, low-volume) sections and employing laser light illumination, both of which serve to decrease sample autofluorescence, and the use of confocal microscopy, which enables precise control of the observed wavelengths as well as sequential image collection. The authors demonstrate the utility of this method in FFPE sections of several human tissues; their high-resolution multicolor images allow characterization of multiple markers in a single sample, and offer sufficient resolution for subcellular antigen localization.

Robertson D, Savage K, Reis-Filho JS, Isacke CM (2008) Multiple immunofluorescence labelling of formalin-fixed paraffin-embedded (FFPE) tissue. *BMC Cell Biol* 9:13–22.

High-throughput screening: Getting patch clamp–quality data from a 1,536-well plate–based ion channel assay. Long QT syndrome is a potentially fatal cardiac condition that is either inherited or acquired as a result of certain drug treatments. Inhibition of the human ether-a-go-go-related gene (hERG) channel has been implicated as a major contributor to acquired long QT syndrome; however, efforts to prescreen drug candidates for hERG channel–inhibiting effects have been hampered by the low throughput or nonfunctional nature of available assays. The authors describe the application of a thallium-based ion flux assay to the problem of obtaining quantitative, high-throughput, and biologically relevant screening information about potential inhibitors of hERG channel activity. The method pairs BacMam-hERG expression with FluxOR™ thallium assay screening to assess compound activity on the hERG channel, using human osteosarcoma cells in a 1,536-well plate format. Their data correctly identified known hERG inhibitors and gave IC_{50} values in line with those previously reported from laborious patch clamp studies.

Titus S, Beacham D, Shahane S, Southall N, Xia M, Huang R, Hooten E, Zhao Y, Shou L, Austin C, Zheng W (2009) A new homogeneous high-throughput screening assay for profiling compound activity on the human ether-a-go-go-related gene channel. *Anal Biochem* 394:30–38.

Neuroscience: Cell proliferation in neurogenic tissue visualized by EdU-based "click" chemistry detection. The identification of proliferating cells is fundamental to gaining insight into the biological processes that underlie neurogenesis. Metabolic incorporation of the thymidine analog BrdU followed by immunofluorescence detection using a labeled anti-BrdU antibody is widely used for identifying dividing cells; however, the technique requires harsh denaturation to allow antibody access, which can result in inconsistent detection with a potential loss of information. The authors investigate the utility of the reactive thymidine analog EdU for the analysis of proliferation *in vitro* as well as in developing brain tissue sections. EdU detection occurs via the "click" reaction, wherein a small fluorescent azide probe is covalently linked to an EdU alkyne group, nullifying the requirement for sample denaturation. They observed intense fluorescence in dividing cells *in vitro*, with the EdU signal remaining localized to the nucleus. Since harsh denaturation wasn't required, EdU-treated neurogenic brain sections retained their native antigenicity, enabling subsequent immunofluorescence analysis of a variety of neuronal markers.

Chehrehasa F, Meedeniya A, Dwyer P, Abrahamsen G, Mackay-Sim A (2009) EdU, a new thymidine analogue for labelling proliferating cells in the nervous system. *J Neurosci Methods* 177:122–130.

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