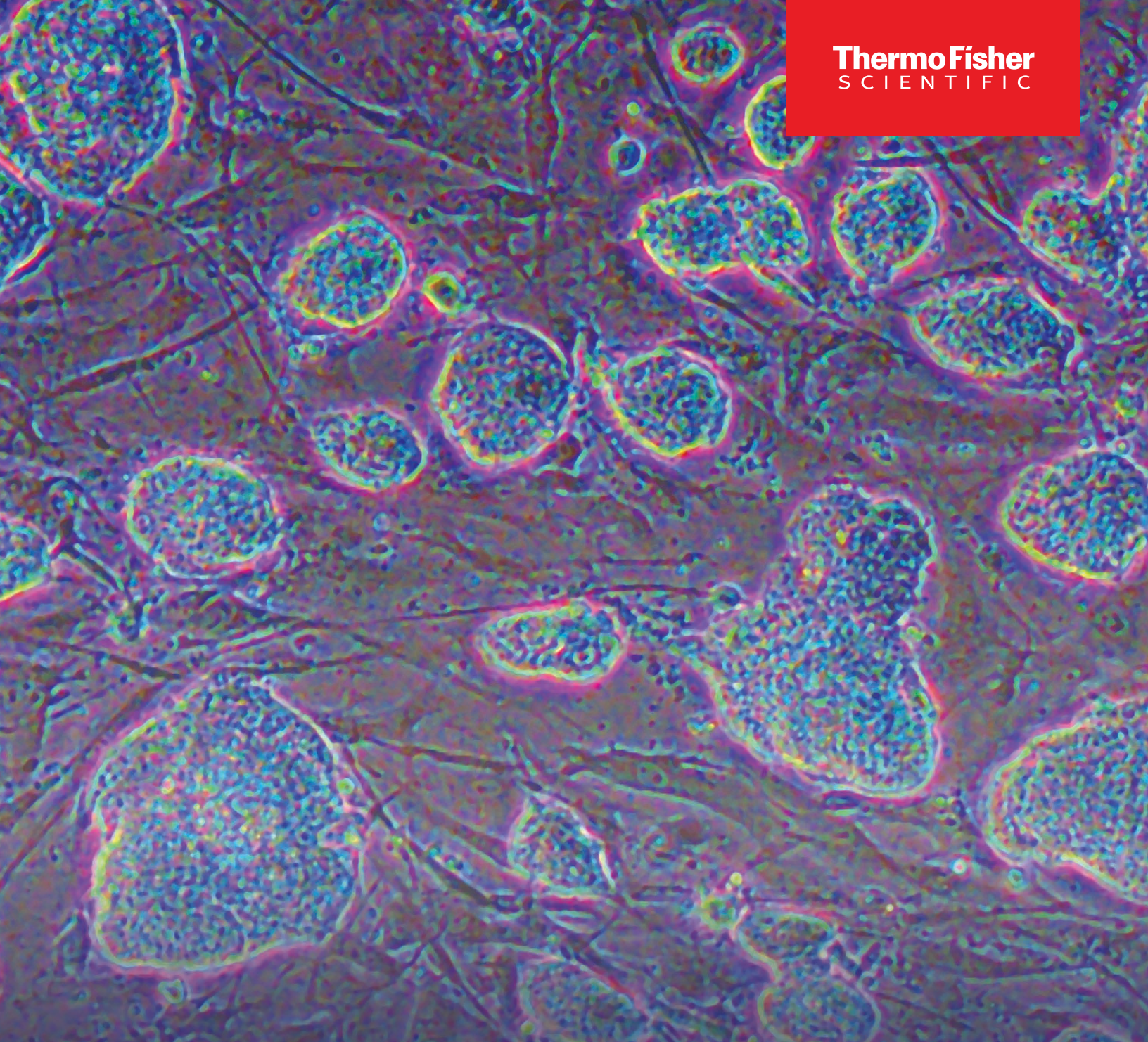


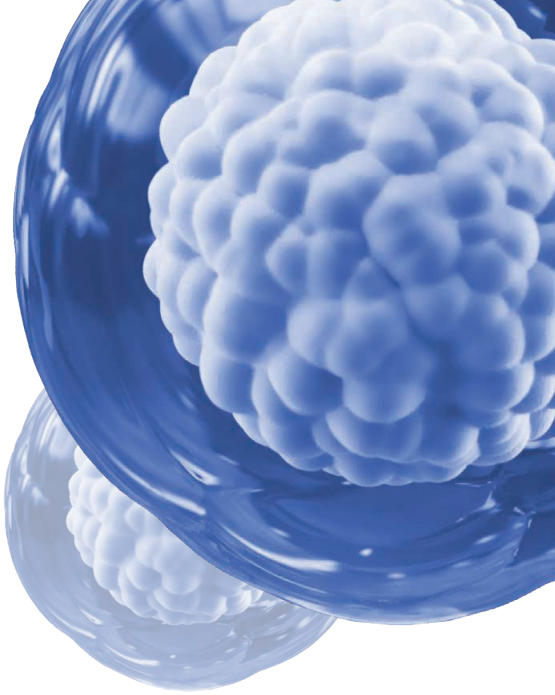
Thermo Fisher SCIENTIFIC

GUIDE TO FEEDER-BASED CULTURE OF PLURIPOTENT STEM CELLS

Make the connection

gibco

Reagents for superior feeder-based PSC culture



Gibco™ reagents cover the full range of products necessary for superior feeder-dependent pluripotent stem cell (PSC) culture, from media and growth factors to cells and passaging enzymes (Figure 1).

Gibco™ KnockOut™ reagents are at the core of this feeder-based PSC culture offering, with basal media optimized for PSC culture and serum replacements that are more defined and reliable than fetal bovine serum (FBS). KnockOut products have been trusted since 1997 and have been cited in over 5,500 PSC-related peer-reviewed publications.

Expand and maintain iPSCs

- KnockOut Serum Replacement
- KnockOut DMEM
- KnockOut DMEM/F-12
- CTS KnockOut SR XenoFree Medium
- CTS KnockOut DMEM
- CTS KnockOut DMEM/F-12

Prepare mouse embryonic fibroblast (MEF) plates

- Mouse Inactivated Embryonic Fibroblasts (iMEFs)
- Attachment Factor Protein
- ESC-qualified FBS

Passage feeder-dependent PSCs

- StemPro Accutase Cell Dissociation Reagent
- TrypLE Express Enzyme
- Trypsin-EDTA
- StemPro EZPassage Disposable Stem Cell Passaging Tool
- Collagenase IV
- Dispase II

Cryopreserve PSCs

- RevitaCell Supplement
- PSC Cryopreservation Kit
- Synth-a-Freeze Medium

Analyze stem cells and their derivatives using a wide range of characterization methods

- Alkaline Phosphatase (AP) Live Stain
- hPSC live imaging kits
- PSC immunocytochemistry kits

Figure 1. Feeder-dependent pluripotent stem cell culture workflow.

KnockOut Serum Replacement

Gibco™ KnockOut™ Serum Replacement (SR) is a more defined, FBS-free culture supplement designed to replace FBS in PSC cultures. It has been cited in more than 4,000 PSC-related publications for over 20 years. For translational applications, Gibco™ CTS™ KnockOut™ SR XenoFree Medium offers comparable performance to KnockOut SR in a xeno-free formulation with associated regulatory documentation.

Benefits of a more defined medium

Serum is a complex mixture of components that can vary widely from lot to lot and can be either beneficial or detrimental to PSCs. Unlike media containing FBS, more defined media have more consistent compositions that reduce the detrimental components and retain the most critical components for PSC maintenance.

The more defined KnockOut SR formulation has been proven more reliable than FBS in both mouse PSC (mPSC) and human PSC (hPSC) culture. It offers:

- More compact colony morphology (Figure 2)
- Better maintenance of undifferentiated PSCs (Figure 3)
- Stable price and stable supply

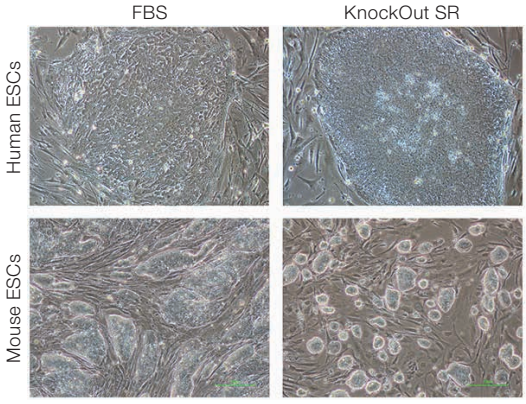


Figure 2. PSC morphology in cultures grown using KnockOut SR vs. FBS. H9 human embryonic stem cells (ESCs) were cultured on mouse embryonic fibroblasts (MEFs) with 20% Gibco™ ESC-qualified FBS or 20% KnockOut SR (top panels). 129S6 mouse ESCs were cultured on MEFs using 15% ESC-qualified FBS or 15% KnockOut SR (bottom panels). Human ESC colonies were more compact when cultured with KnockOut SR than with ESC-qualified FBS. Mouse ESCs cultured with KnockOut SR were dome-shaped and more compact than with ESC-qualified FBS.

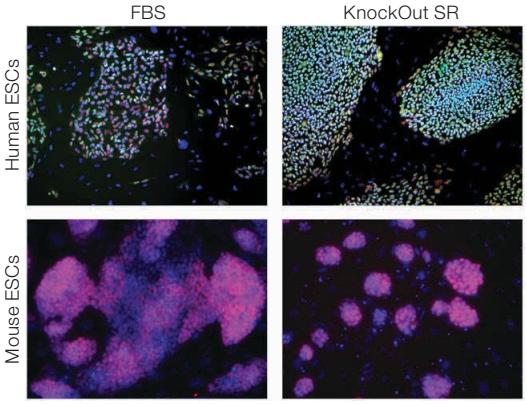


Figure 3. PSC marker expression in cultures grown with KnockOut SR vs. FBS. H9 human ESCs and 129S6 mouse ESCs were grown on MEFs under the same conditions described in Figure 2. (Top panels) Human ESCs grown using KnockOut SR exhibit uniformly high Oct4 (red) and Sox2 (green) expression, while those grown using ESC-qualified FBS are heterogeneous and show dimmer Sox2 staining. (Bottom panels) Mouse ESCs grown using KnockOut SR also show higher and more homogeneous expression of Oct4 than those cultured in ESC-qualified FBS. DAPI (blue) was used as a nuclear stain.

Feeder-dependent culture proven more reliable than FBS

Choose your KnockOut product combination

Mouse PSC culture for research use

Recommended products:

- KnockOut SR
- Gibco™ KnockOut™ DMEM
- Gibco™ LIF Recombinant Mouse Protein

Maintain mPSCs with KnockOut SR and a basal medium that approximates the low osmolarity of mouse embryonic tissue.

- **Minimizes differentiation**—improves maintenance of undifferentiated mPSCs compared to FBS (Figure 4) and traditional DMEM
- **Maintains quality colonies**—colonies are more dome-shaped and show more homogeneous gene expression compared to FBS-based medium
- **Easy to transition into**—supports cultures that are directly thawed or passaged from FBS-based medium

Human PSC culture for research use

Recommended products:

- KnockOut SR
- Gibco™ DMEM/F-12, GlutaMAX™ supplement
- Gibco™ bFGF Recombinant Human Protein

Culture hPSCs confidently with KnockOut SR, a more defined medium that is proven more reliable than FBS for basic research applications.

- **Supports robust growth**—results in a higher growth rate compared to FBS (Figure 5)
- **Maintains quality colonies**—colonies are more compact and show strong expression of PSC markers compared to FBS
- **Easy to transition into**—supports cultures that are directly passaged from FBS-based medium
- **Stable price and supply**—FBS-free formulation is not subject to the same FBS price and supply fluctuations

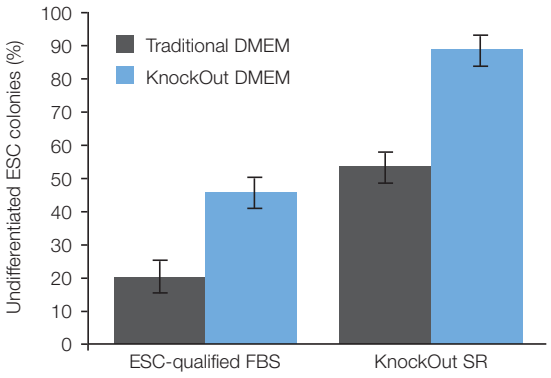


Figure 4. Mouse PSC culture with KnockOut SR vs. FBS in the absence of LIF. Mouse D3 ESCs were cultured at low density in either Gibco™ DMEM (with high glucose) or KnockOut DMEM, supplemented with either ESC-qualified FBS or KnockOut SR. No LIF was used. After 7 days, colonies were fixed and stained for alkaline phosphatase, a marker for undifferentiated ESCs. Undifferentiated colonies were scored based on morphology and staining.

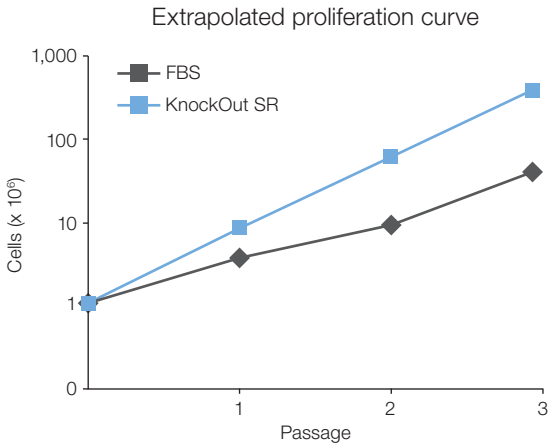


Figure 5. Human PSC growth with KnockOut SR vs. FBS. H9 human ESCs were grown on MEFs under the same conditions as in Figure 2, and the mean viable cell numbers were plotted to growth curves for media supplemented with ESC-qualified FBS or KnockOut SR. Proliferation of human ESCs was significantly higher in KnockOut SR over 3 passages.

Xeno-free human PSC culture for translational use

Recommended products:

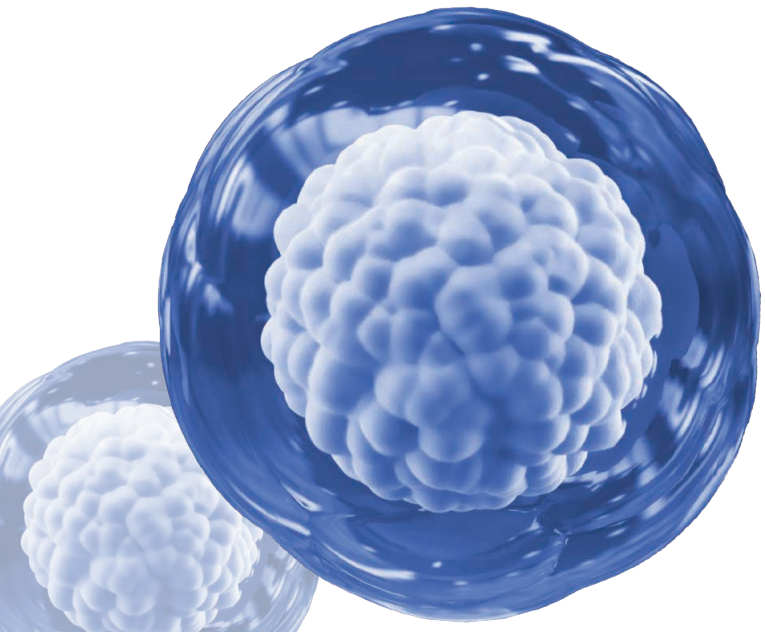
- CTS KnockOut SR XenoFree Medium
- Gibco™ CTS™ KnockOut™ DMEM
- Gibco™ FGF-Basic Full Length Recombinant Human Protein
- Gibco™ CELLstart™ Substrate

Count on Gibco™ Cell Therapy Systems (CTS™) products for the xeno-free derivation, maintenance, and cryopreservation of human PSCs in translational applications (Figure 6).

- **Xeno-free**—contains only recombinant or human-derived material and is compatible with human feeder cells
- **Provides extensive QC testing**—helps reduce the burden of qualifying material when transitioning to clinical applications
- **Provides traceability documentation**—supporting documentation available for regulatory submissions

Table 1. Comparison of FBS and the KnockOut SR formulation.

	Species	Serum	KnockOut SR	References
Unwanted differentiation	Human, mouse	Higher	Lower	1–5
Efficiency of ESC derivation	Human, mouse	Lower	Higher	3, 6–8
Clonal efficiency	Human, mouse	Lower	Higher	7, 9



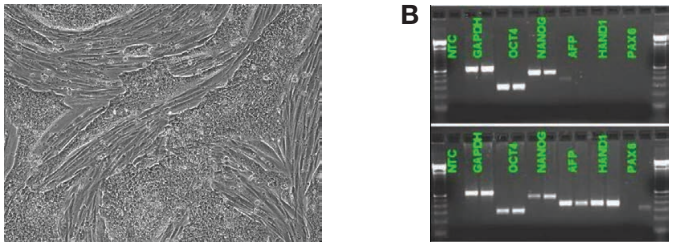


Figure 6. Xeno-free culture and gene expression of hESCs. **(A)** Morphology of BG01v hESCs at passage 4, cultured in CTS KnockOut SR XenoFree Medium on human foreskin fibroblast (HFF) feeder cells attached with CELLstart Substrate. **(B)** Maintenance of pluripotency with CTS KnockOut SR XenoFree Medium. Following 10 passages, either in KnockOut SR (left lane of the pair for each marker) or in CTS KnockOut SR XenoFree (right lane for each marker) on HFFs attached with CELLstart Substrate, undifferentiated BG01v gene expression was examined by RT-qPCR of pluripotency markers, OCT4 and NANOG (top). Gene expression of embryoid bodies generated from the same passage-10 BG01v/HFF culture (bottom). (NTC=No template control; GAPDH=reference gene).

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Complete your feeder-dependent culture workflow

Prepare mouse embryonic fibroblast (MEF) plates

- **Gibco™ Mouse (ICR) Inactivated Embryonic Fibroblasts (iMEFs)**—prepare a feeder layer you can trust with these cryopreserved γ-irradiated MEFs that are tested for post-thaw viability, growth arrest, and ability to support mouse and human PSCs
- **Gibco™ Attachment Factor Protein**—facilitate the attachment and spreading of iMEFs using this convenient, ready-to-use 0.1% gelatin substrate
- **Gibco™ ESC-qualified FBS**—grow iMEFs with this specialty serum that is specifically tested for supporting PSC growth, so it should not interfere with subsequent feeder-dependent PSC culture; ESC-qualified FBS is also another option for culturing feeder-dependent mouse PSCs (Figure 7)

Passage feeder-dependent mouse PSCs

- **Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent**—gently passage mPSCs using this ready-to-use solution of proteolytic and collagenolytic enzymes, which does not contain any mammalian or bacterially derived material
- **Gibco™ TrypLE™ Express Enzyme**—gently dissociate mPSCs with this ready-to-use, room temperature–stable recombinant enzyme
- **Gibco™ Trypsin-EDTA**—dissociate mPSCs with this strong and versatile mixture of proteases from porcine pancreas

Passage feeder-dependent human PSCs

- **Gibco™ StemPro™ EZPassage™ Disposable Stem Cell Passaging Tool**—quickly and consistently obtain the ideal hPSC cluster size by performing mechanical passaging with this roller tool (Figure 8)
- **Gibco™ Collagenase IV**—passage hPSCs using this gentle enzyme, which requires longer incubation
- **Gibco™ Dispase II**—passage hPSCs using this enzyme for a shorter incubation

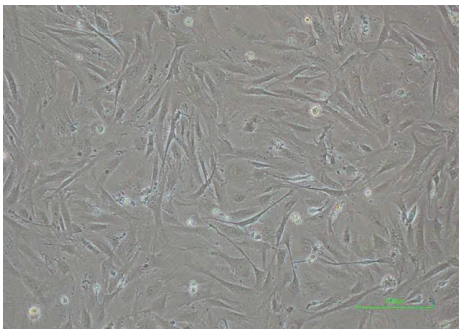
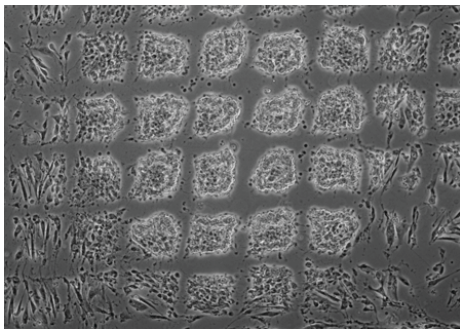


Figure 7. iMEFs cultured on Attachment Factor Protein with DMEM and ESC-Qualified FBS.

A



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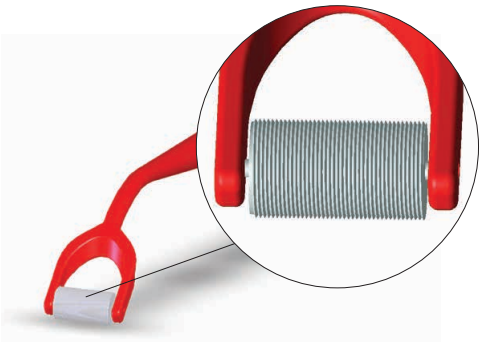

Figure 8. (A) Feeder-dependent hPSC colony after being cut with the StemPro EZPassage tool shown in (B).

Cryopreserve human PSCs

- **Gibco™ RevitaCell™ Supplement**—improve the post-thaw viability and growth of human PSCs with this optimized supplement containing a high-specificity ROCK inhibitor, antioxidants, and free radical scavengers (Figure 9)
- **Gibco™ PSC Cryopreservation Kit**—freeze and recover human PSCs with this combination of a ready-to-use, xeno-free PSC cryopreservation medium and RevitaCell Supplement to maximize post-thaw recovery and minimize unwanted differentiation
- **Gibco™ Synth-a-Freeze™ Cryopreservation Medium**—freeze human PSCs with this defined, ready-to-use cryopreservation medium for improved cell viability and recovery after thawing

Characterize human PSCs

- **Invitrogen™ Alkaline Phosphatase (AP) Live Stain**—quickly identify PSCs with this fluorescent enzyme substrate that reversibly stains AP-expressing cells while maintaining cell viability (Figure 10)
- **Invitrogen™ hPSC live imaging kits**—visualize the positive and negative PSC markers TRA-1-60 and CD44, respectively, in live cells using these superior kits that pair optimally labeled primary antibodies with a reduced-background imaging medium (Figure 11)
- **Invitrogen™ PSC immunocytochemistry kits**—analyze up to four key PSC markers (OCT4, SOX2, SSEA4, and TRA-1-60) with these high-performance kits, which include a complete set of primary and secondary antibodies, a nuclear DNA stain, and premade buffers

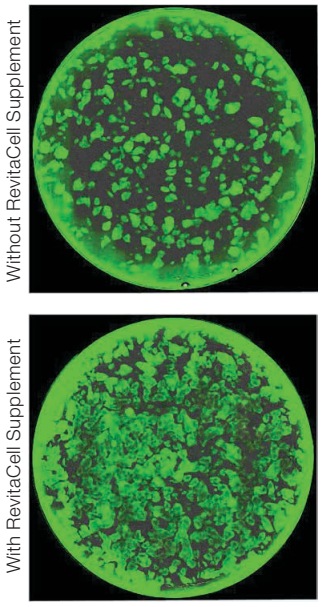

Figure 9. RevitaCell Supplement boosts post-thaw recovery of human PSC colonies on feeders. Whole-well images show OCT4 staining of colonies (green) 6 days post-thaw.

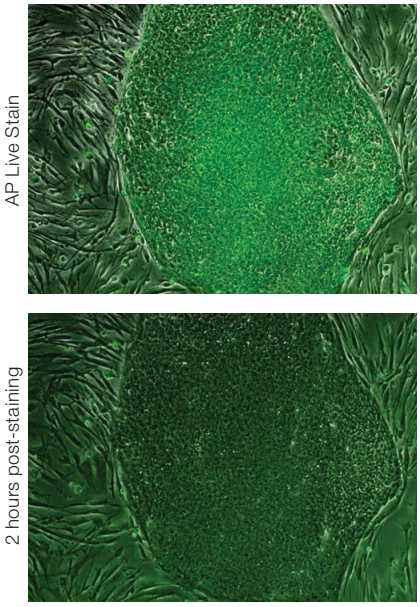

Figure 10. Live AP staining is reversible. AP Live Stain (green) shows robust staining of a human PSC colony. The fluorescent signal is lost from the cells within 90 minutes after removal of the dye from the medium.

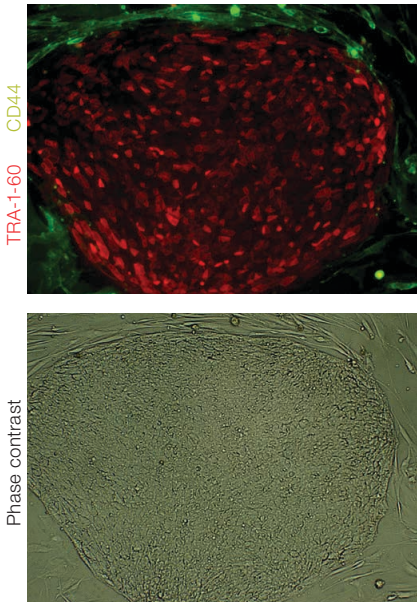

Figure 11. Live-cell imaging of human PSCs using the Invitrogen™ TRA-1-60 Alexa Fluor™ 555 and Invitrogen™ CD44 Alexa Fluor™ 488 Conjugate Kits. Imaging was performed with Gibco™ FluoroBrite™ DMEM imaging medium.

Ordering information

Product	Cat. No.
Mouse PSC culture for research use	
Knockout SR Medium	10828010 or 10828028
KnockOut DMEM	10829018
Leukemia Inhibitory Factor (LIF) Recombinant Mouse Protein	PMC9484
Passaging mouse PSCs	
StemPro Accutase Cell Dissociation Reagent	A1110501
TrypLE Express Enzyme (1X), no phenol red	12604021
Trypsin-EDTA (0.05%), phenol red	25300054
Human PSC culture for research use	
Knockout SR Medium	10828010 or 10828028
DMEM/F-12, GlutaMAX Supplement	10565018
Xeno-free human PSC culture for translational use	
CTS KnockOut SR XenoFree Medium*	12618012 or 12618013
CTS KnockOut DMEM*	A1286101
CELLstart Substrate*	A1014201
Preparing MEF plates	
Mouse (ICR) Inactivated Embryonic Fibroblasts	A24903
Attachment Factor Protein	S006100
FBS, embryonic stem cell–qualified	10439024 or 16141061
DMEM, high glucose, GlutaMAX Supplement, pyruvate	10569010

Product	Cat. No.
Passaging human PSCs	
StemPro EZPassage Disposable Stem Cell Passaging Tool	23181010
Collagenase, Type IV, powder	17104019
Dispase II, powder	17105041
Cryopreserving human PSCs	
RevitaCell Supplement (100X)	A2644501
PSC Cryopreservation Kit	A2644601
Synth-a-Freeze Cryopreservation Medium	A1254201
Characterizing human PSCs	
Alkaline Phosphatase Live Stain	A14353
TRA-1-60 Kits for Live Cell Imaging (Alexa Fluor 488, 594, or 555)	A25618, A24882, or A24879
CD44 AlexaFluor 488 Conjugate Kit for Live Cell Imaging (rat anti-human/mouse mAb)	A25528
Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit	A24881
Pluripotent Stem Cell Immunocytochemistry Kit (OCT4, SSEA4)	A25526

* For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. CAUTION: Not intended for direct administration into humans or animals.

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