CellEvent™ Senescence Green Flow Cytometry Assay Kit

Catalog Numbers C10840, C10841

Pub. No. MAN0018281 **Rev.** B.0

Contents and storage

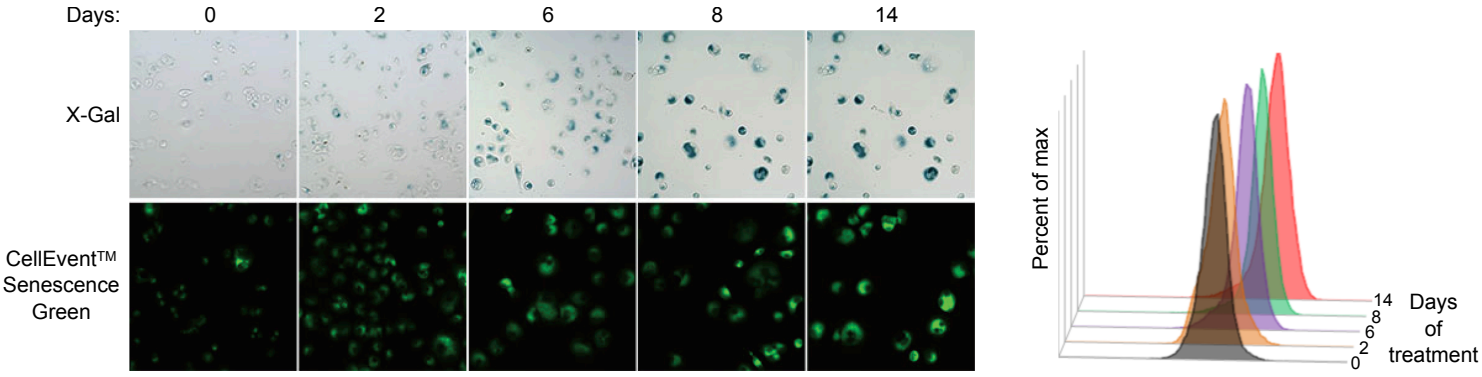
Component	C10840 (50 assays)	C10841 (200 assays)	Storage[1]
CellEvent™ Senescence Green Probe (1000X)	1 × 50 µL (in DMSO)	4 × 50 µL (in DMSO)	• 2–8°C • Protect from light
CellEvent™ Senescence Buffer	1 × 50 mL	4 × 50 mL	2–8°C
Approximate fluorescence excitation and emission maxim: 490 nm/514 nm; Flow cytometry channel and laser settings for fluorescein are recommended.			

[1] When stored as directed, the product is stable for at least 6 months.

Product description

The Invitrogen™ CellEvent™ Senescence Green Flow Cytometry Assay Kit provides the CellEvent™ Senescence Green Probe and an optimized buffer for the detection of senescent cells in fixed samples. The assay can be multiplexed with antibodies against surface and intracellular markers, along with other fluorescent reagents compatible with paraformaldehyde fixation.

Detection of ß-Galatosidase Activity in Cells Upon Induction of Senescence Over Time



(A) (B)

T47D human epithelial cells were left untreated (day 0) or treated with 5 µM palbociclib in media every other day for 15 days to induce senescence (days 2–14) through cyclin D checkpoint blockade. (A) Cells were stained using x-gal or the CellEvent™ Senescence Green Probe. (B) A parallel set of samples where cells were treated identically to induce senescence were trypsinized, resuspended in 1X PBS, and fixed in 4% paraformaldehyde for 10 minutes at room temperature, then stained with the CellEvent™ Senescence Green Probe diluted 1/1000 in CellEvent™ Senescence Buffer for 90 minutes in a 37°C incubator with no CO_2. Cells were washed in 1X PBS with 1% BSA, then resuspended in 1X PBS. Data was acquired on an Invitrogen™ Attune™ NxT instrument using a 488-nm laser. Emission was collected using a 530/30-nm filter. An increase in senescence-associated ß-galactosidase expression, a hallmark for the onset of senescence, is detected by the CellEvent™ Senescence Green Probe.

About senescence detection

Normal cells are known to have a limited replicative lifespan commonly known as the Hayflick limit.[1] After normal cells enter cell cycle arrest, they enter into a senescence phase where they remain metabolically active without undergoing cell death processes. These senescent cells adopt a specific phenotypic state that includes the appearance of multinucleated cells, increased vacuolization, expression of pH-dependent β-galactosidase, and morphological changes where cells become enlarged and extended.[2,3]

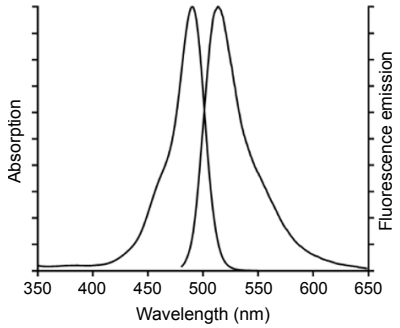
Senescence, through various mechanisms, can also play a role in tumor suppression, tumour progression, aging, and tissue repair. For example, in the tumor suppressive and aging pathways, telomeres shorten until the Hayflick limit is reached. In response, stress stimuli that are associated with tumor suppressing properties, such as the oncogenes Ras, cyclin E, E2F3, and Raf are expressed.[4] As part of the DNA damage response pathway, one sees p53/p21 activation.[5,6] Lastly, tumor progression is helped by the development of the senescence-associated-secretory phenotype (SASP) that turns senescent fibroblasts into proinflammatory cells, resulting in increased expression of proteins such as IL-6.[7]

Activation of β-galactosidase enzyme (β-gal) is commonly used as a biomarker for senescent cells. Although β-gal activity is considered a hallmark of senescence, it does not cause the phenomena. Rather, it has been shown that there is an increase in enzymatic activity as the cells enter a senescent state. This hydrolase enzyme resides in lysosomes and converts β-galactosides into monosaccharides under acidic pH conditions. The activity is optimal under lysosomal pH 4, but conventional assays measure at pH 6. It has been shown that normalized β-gal activity is twice as high in senescent cells as in presenescent cells, regardless of the pH value used for testing.[8]

The colorimetric substrate for β-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, known as x-gal, has long been used to detect metabolic activity in cells *in vitro*. On hydrolysis by β-gal, x-gal converts to a blue precipitate, which is not appropriate for flow cytometric assays. Therefore, we have developed a sensitive, fluorescent substrate for β-gal that can be used in flow cytometry assays for the detection of senescent cells. The CellEvent™ Senescence Green Probe is a fluorescent-based reagent that contains two galactoside moieties, making it specific to β-galactosidase. The enzyme-cleaved product is retained in the cell due to covalent binding of intracellular proteins and emits a fluorogenic signal that has absorption/emission maxima of 490/514 nm.

The Invitrogen™ CellEvent™ Senescence Green Flow Cytometry Assay Kit provides the CellEvent™ Senescence Green Probe and buffer for the detection of senescent cells in fixed samples based on the provided protocol. This assay can also be multiplexed with antibodies against surface and intracellular markers, along with other fluorescent reagents compatible with paraformaldehyde fixation.

Fluorescence excitation and emission spectra of CellEvent™ Senescence Green Probe



Approximate fluorescence excitation and emission maxim is 490 nm/514 nm.

Procedural guidelines

- The staining procedure was developed using adherent T47D cells, a ductal carcinoma cell line, that were trypsinized before staining, but the procedure can be adapted for any cell type.
- Growth medium, cell density, cell type variations, and other factors can influence staining.
- In initial experiments, test a range of stain concentrations to determine the optimal stain concentration for the given cell type, buffer, and experimental conditions.

Before you begin

- Prepare Fixation Solution by diluting a stock solution of paraformaldehyde to 2% in PBS.

  Note: We recommend starting with 2% paraformaldehyde in PBS. If optimization is required, increase the amount of paraformaldehyde to a maximum of 4%.

- Prepare Working Solution: Warm the CellEvent™ Senescence Buffer to room temperature. Dilute the CellEvent™ Senescence Green Probe (1,000X) into the pre-warmed CellEvent™ Senescence Buffer. Vortex to mix well before adding to cells. Working Solution should be prepared at time of staining.

  Note: Working concentration can be cell type and cell concentration dependent. We recommend starting with a dose response curve ranging from 1:500–1:4000 dilution of the CellEvent™ Senescence Green Probe for 5×10^5 cells/100 µL.

Stain cells

The following protocol requires that cells be in suspension. Adherent cell lines may be used but must be trypsinized to create a single cell suspension after induction of senescence.

1. *(Optional)* Induce senescence in cells using the desired method.

   Note: Prepare a negative control by incubating cells in the absence of the senescence inducing agent.

2. Wash the cells, then resuspend them in 1X PBS to a concentration of 0.5×10^6–1.0×10^6 cells per 100 µL.

3. Aliquot 100 µL of the cell suspension to flow tubes.

4. Centrifuge the tubes, then discard the media.

5. *(Optional)* Stain cell surface antigens with antibodies.
   a. Resuspend the cells in 100 µL of 1% BSA in PBS or appropriate staining buffer.
   b. Add surface antigens, then mix well.
   c. Incubate for the recommended time and temperature, protected from light.
   d. Wash the cells in 1% BSA in PBS, centrifuge at 400 × *g* for 5 minutes, then remove the supernatant.

6. Resuspend the cells in 100 µL of Fixation Solution.

7. Incubate for 10 minutes at room temperature, protected from light.

8. Wash the cells in 1% BSA in PBS to remove the Fixation Solution.

9. Resuspend the cells in 100 µL of Working Solution.

10. Incubate the cells for 1–2 hours at 37°C without CO_2, protected from light.

    IMPORTANT! Do not incubate the cells in the presence of CO_2, because CO_2 changes the pH of the reaction.

11. After incubation, remove the Working Solution, then wash the cells with 1% BSA in PBS.

    Note: Cells can be permeabilized with 0.25% Triton X-100 or 0.1% saponin after staining. Do not permeabilize cells before staining with the probe. You can store the cells overnight at 4°C, protected from light.

12. *(Optional)* Stain intracellular antigens with antibodies.
    a. Resuspend and permeabilize the cells with 100 µL of 0.25% Triton X-100 in 1% BSA in PBS and incubate for 15 minutes at room temperature. Protect from light.
    b. Wash the cells in 1% BSA in PBS, then centrifuge at 500 × *g* for 5 minutes. Remove and discard the media.
    c. Resuspend the cells in 100 µL of 1% BSA in PBS or other appropriate staining buffer.
    d. Add antibodies against the intracellular antigens of choice, mix well, then incubate for the recommended time and temperature. Protect from light.
    e. Wash the cells in 1% BSA in PBS, centrifuge at 500 × *g* for 5 minutes, then remove the supernatant.

13. Resuspend the cells in 1% BSA in PBS or FACS buffer, then analyze on a flow cytometer using a 488-nm laser and 530-nm/30 filter or similar.

References

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