CellEvent™ Senescence Green Detection Kit

Catalog Numbers C10850, C10851

Pub. No. MAN0018221 **Rev.** A.0

Contents and storage

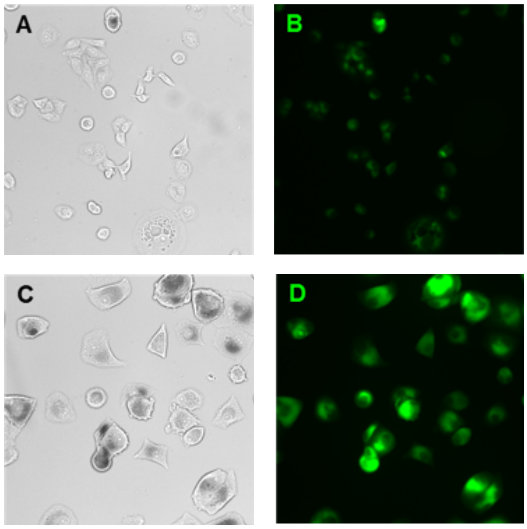
Component	C10850	C10851	Storage[1]
CellEvent™ Senescence Green Probe (1000X)	1 × 25 µL (in DMSO)	1 × 100 µL (in DMSO)	• 2–8°C • Protect from light
CellEvent™ Senescence Buffer	1 × 50 mL	2 × 50 mL	2–8°C
Approximate fluorescence excitation and emission maxim: 490 nm/514 nm; Alexa Fluor™ 488/FITC filter sets are recommended.			

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

Product description

The Invitrogen™ CellEvent™ Senescence Green Detection Kit provides the CellEvent™ Senescence Green Probe and an optimized buffer for the detection of senescent cells in fixed samples. The assay can also be multiplexed with other fluorescent reagents compatible with paraformaldehyde fixation.

Senescent and non-senescent cells treated with CellEvent™ Senescence Green Probe or X-Gal



T47D cells treated with 5 µM palbociclib via media changes every other day for 15 days to induce senescence through cyclin D checkpoint blockade (senescent) and untreated T47D cells (non-senescent) and fixed in 4% paraformaldehyde for 10 minutes at room temperature, then stained with either CellEvent™ Senescence Green Probe or X-Gal for 90 minutes in a 37°C incubator with no CO_2. Panels A and C were stained with X-Gal; Panels B and D were stained with the CellEvent™ Senescence Green Probe.

About senescence detection

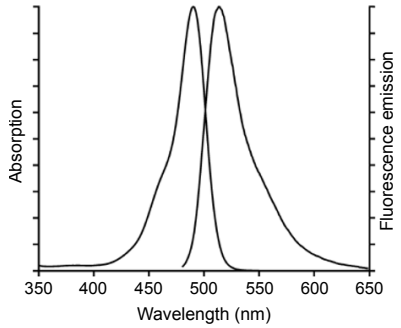
Due to limited replicative lifespan, normal cells enter cell cycle arrest, also known as cellular senescence[1]. While in this senescence phase the cells remain metabolically active without undergoing cell death or division. These senescent cells adopt a specific phenotypic state that includes the appearance of multinucleated cells, increased vacuolization, expression of pH-dependent β-galactosidase, and morphological changes where cells become enlarged and extended[2, 3]. Senescence, through a variety of mechanisms, can also play a role in tumor suppression, tumor progression, aging, and tissue repair.

Activation of β-galactosidase enzyme (β-gal) is commonly used as a biomarker for senescent cells. 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 for the detection of senescence measure this activity 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[4].

The colorimetric substrate for β-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), is used to detect metabolic activity and senescence activity in cells *in vitro*. β-gal hydrolyzes X-Gal to a blue precipitate that is detected using traditional light microscopy techniques. However, this colorimetric probe is limited due to inconsistent signal, length of assay, and inability to multiplex.

We developed a sensitive, fluorescent substrate for β-gal that can be used 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 within the cell due to covalent binding of intracellular proteins and emits a fluorogenic signal that has excitation/emission maxima of 490/514 nm.

Fluorescence excitation and emission spectra of CellEvent™ Senescence Green Probe



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

Required materials not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Incubator with CO_2	MLS
Incubator without CO_2	MLS
37°C water bath	MLS
Adjustable micropipettors	MLS
Flat-bottom 96-well plates	MLS
Plastic film	MLS
1% bovine serum albumin (BSA) in phosphate buffered saline (PBS), pH 7.1–7.4	MLS
Buffered saline solution such as PBS, D-PBS or TBS	MLS
2–4% paraformaldehyde	MLS

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 37°C. Dilute the CellEvent™ Senescence Green Probe (1,000X) into the pre-warmed CellEvent™ Senescence Buffer. For each well of a 96-well plate 100 µL of Working Solution will need to be prepared.

Stain adherent cells

1. Plate cells into a flat-bottom 96-well plate, then allow for attachment by overnight incubation.
2. Treat cells with the appropriate inducer of senescence for the desired time.
3. Wash wells with PBS.
4. Fix the cells by adding 100 µL/well of Fixation Solution for 10 minutes at room temperature. Protect from light.
5. Wash cells with 100 µL of 1% BSA in PBS per well to remove the Fixation Solution.
6. Add 100 µL/well of the prewarmed Working Solution per well of the 96-well plate.
7. Cover the plate with plastic film to prevent moisture loss, then incubate for 2 hours at 37°C without CO_2. Protect from Light.

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

8. After incubation, remove the Working Solution, then wash the wells 3 times with 100 µL of PBS per well.
9. Add 100 µL of PBS per well, then image using an Alexa Fluor™ 488/FITC filter set.

References

1. Hayflick L: The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 1965; 37:614-636
2. Collado M, Serrano M. Senescence in tumors: evidence from mice and humans. Nat Rev Cancer 2010: 10:51-57
3. Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. Genes Dev 2001: 24:2463-2479
4. Gary R, Kindell SM. Anal Biochem. 2005; 343:329-334.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.