

DATA SHEET

Version: 03
Revision date: 15/03/2023

1. Identification

Product name

Senescence Detection Kit (SA- β -gal Staining)

100 assays

Cat. No:**CA090**

2. Description

Senescence is one of the most fundamental aspects of cell behaviour and is thought to play a critical role in regulating cellular lifespan both in vitro and in vivo. The cell culture in vitro after a period of rapid proliferation, cell division rate slows, and ultimately ceases altogether, with the cells becoming unresponsive to mitogenic stimuli. This process is called Senescence. The senescence cells display a phenotype like increase of cell size, distinctive flat morphology, changes in gene expression and activity of senescence-associated β -galactosidase (SA- β -gal).

Senescence represents tumour suppressor mechanism for this reason cellular senescence has become an increasingly target in the development of novel therapeutics.

Senescence detection kit measures activity of SA- β -Gal in cells cultures by hydrolysis of X-gal (5-Bromo-4-chloro-3-indolyl β -D-galactosidase), which results in the accumulation of a distinctive blue colour in senescent cells. The SA- β -Gal is present only in senescent cells and is not found in pre-senescent, quiescent, or immortal cells.

3. Composition

Item	Quantity
X-Gal	150 mg
PBS (10X)	50 ml
10X Fixative solution*	15 mL
5X Staining Solution	15 mL
100X Staining Solution Supplement A*	1 x 1.5 mL
100X Staining Solution Supplement B*	1 x 1.5 mL

! Caution



* The **fixative solution** contains formaldehyde and glutaraldehyde, which are toxic and corrosive solutions. Wear personal protective clothing when handling solutions and use in a fume hood.

* **Staining solution supplements (A and B)** contains $K_4[Fe(CN)_6] \cdot 3H_2O$ and $K_3[Fe(CN)_6]$, which are irritants for humans and dangerous for the environment. Wear personal protective clothing (e.g., nitrile or latex gloves, lab coat and goggles) when handling solution and discard in an appropriate manner.



DATA SHEET

Version: 03
Revision date: 15/03/2023

4. Storage specifications


Store X-Gal (150 mg, Lyophilized) at -20°C, protected from light. Store reconstituted X-Gal at -20°C.
10X Fixative Solution, 5X Staining Solution and 100X Staining Supplement (A and B) can be stored at +4°C. If precipitation occurs, simply warm up the solution to 37°C to solubilize the precipitates.

5. Applications

Detect SA-β-Galactosidase activity, a known characteristic of senescent cells, in cultured cell and tissue sections.

6. Reagent preparation:

1. Prepare X-Gal solution: Dissolve 20 mg in 1 ml DMF (N, N-dimethylformamide) to prepare a 20X stock solution. This solution must be freshly prepared or can be stored for 2 weeks at -20°C.

!Caution : Dimethylformamide is toxic and harmful. Wear personal protective clothing when handling solution. Use a fume hood.
Always use a polypropylene container or glass to make and store the X-gal. Do not use polystyrene.

2. Prepare 1X Fixative Solution: Prepare a 1X Fixative Solution by diluting the provided 10X stock 1:10 in water. Store the diluted solution at room temperature for up to six months.

3. Prepare 1X Staining Solution: Prepare a 1X Staining Solution by diluting the provided 5X stock 1:5 in water. Store the diluted solution at room temperature for up to six months.

4. 1XPBS: To make 500 mL of 1XPBS, add 50 mL of 10X PBS to 450 mL of water. Store the 1X solution at room temperature.

7. Recommended Protocol

The following protocol is designed for each well in a 6-well plate (∅ 35 mm) and may be modified accordingly to suit other culture plate sizes.

1. Remove culture medium and wash cells once with 2 mL of **1X PBS**.
2. Fix the cells or frozen tissue sections: Add enough **1X fixative solution** to submerge the cells (1–2 ml per well). Incubate for 10–15 minutes at room temperature.
3. While the cells are in the Fixative Solution, prepare the **Staining Solution Mix** using a polypropylene plastic tube only.
For each well, prepare:
 - 1X Staining Solution: 697.5 µL
 - 100X Staining Supplement A: 7.5 µL
 - 100X Staining Supplement B: 7.5 µL
 - 20 mg/ml X-gal in DMF: 37.5 µL

The pH of Staining Solution Mix must be at 6.0. pH differences can affect staining; A low pH can result in false positives and high pH can result in false negatives. If necessary, use HCl or NaOH to lower or raise pH, respectively. The increased activity of SA-β-galactosidase is usually detected at pH 6 and constitutes the basis of the Senescent Cell Detection assay. Use a positive control in case there are no senescent cells in your conditions.



4. Remove the fixation solution and wash the cells twice with 2 ml of **1X PBS**.
5. Add 750 μ L of the **Staining Solution Mix** to each well. Cover the plate to prevent evaporation. Incubate plate in the dark at 37°C (2 hour – overnight incubation).

Do not incubate the cells in a CO₂ incubator. CO₂ levels found in general 37°C incubators will lower the pH of the staining solution thereby affecting the color development.

6. Observe the cells under a microscope for development of blue color. Count the blue stained senescence cells and the total number of cells. Calculate the percentage of cells expressing β -galactosidase (senescent cells).

Blue color is detectable in some cells within 2 h, but staining is maximal after 12–16 h. The exact incubation time must be optimized. Cells may be observed every 4 h during the first 12 h, and subsequently every 12 h.

8. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. **Canvax Reagents S.L.U.** shall not be held liable for any damage resulting from handling or from contact with the above product.

