Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

Version: 04
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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 aperiod 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 Suplement A*	1 x 1.5 mL
100X Staining Solution Suplement 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 $K_4[Fe(CN)6]\ 3H_2O$ 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.

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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 storedat +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.

For frozen tissue sections, standard cryosectioning procedures should be performed prior to staining. Tissue handling, section thickness and fixation conditions may require minor optimization depending on the tissue type.

1. For cultured cells: Remove culture medium and wash cells once with 2 mL of 1X PBS.

   For frozen tissue sections: Place the frozen tissue sections on microscope slides. If necessary, gently rinse the sections with 1X PBS before fixation.

2. Fix the cells or frozen tissue sections: Add sufficient **1X Fixative Solution** to completely cover the sample. For cultured cells in a 6-well plate, 1–2 mL per well is typically sufficient. Incubate for 10–15 minutes at room temperature.

3. While the samples are in the Fixative Solution, prepare the **Staining Solution Mix** using a polypropylene plastic tube only.

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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 the Staining Solution Mix must be 6.0. Variations in pH may affect staining results: lower pH values may produce false positives, whereas higher pH values may lead to false negatives. If necessary, adjust the pH using HCl or NaOH. The increased activity of SA-β-Galactosidase detected at pH 6.0 forms the basis of the Senescence Detection assay. A positive control is recommended when no senescent cells are expected under the experimental conditions.

4. Remove the fixation solution and wash the samples twice with 2 mL of **1X PBS**.

5. Add sufficient **Staining Solution Mix** to completely cover the samples. For cultured cells in a 6-well plate, approximately 750 µL per well is recommended. Cover the samples to prevent evaporation. Incubate at 37°C (2 hour – overnight incubation).

Do not incubate the samples in a CO_2 incubator. CO_2 levels found in general 37°C incubators will lower the pH of the staining solution thereby affecting the color development.

6. Observe the stained samples under a bright-field microscope for the development of blue staining. For cultured cells, the percentage of senescent cells may be determined by counting blue-stained and total cells. For tissue sections, staining should be evaluated using appropriate histological analysis methods.

Blue staining may become detectable within 2 hours, although maximum staining is typically observed after 12–16 hours. The optimal incubation time should be determined experimentally for each sample type.

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.
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