β-Gal Senescence Staining Kit

Catalog No.: RA20075

Basic Information

Product name	β-Gal Senescence Staining Kit
Sizes	100T
Storage	-20℃, keep away from light
Shipping	Shipped with ice pack
Validity	12 months

Product Introduction

Cell senescence refers to the gradual decline in cell proliferation, differentiation capacity, and physiological functions that occur over time during life activities. Under normal circumstances, the body eliminates senescent cells, while those remaining may accumulate, potentially causing low-level inflammation or further inducing tissue degeneration and carcinogenesis. Key characteristics of cellular senescence include morphological changes, cell cycle arrest, senescence-associated secretory phenotype, macromolecular damage, and metabolic disorders. Currently, the most specific marker for identifying senescence is senescence-associated β-galactosidase (SA-β-Gal), which accumulates with cellular aging and is absent in pre-senescence cells, quiescent cells, and terminally differentiated cells. This kit utilizes the fact that β-Gal in senescent cells can hydrolyze 5-bromo-4-chloro-3-indolyl-β-D-pentose galactoside (X-gal) into a blue substance at pH 6.0, enabling observation of cellular or tissue senescence under optical microscopy.

Scope of Application

Cell aging detection

Product features

Effect is obvious: blue substance is obvious, convenient to observe;

good stability: can withstand 20 times of repeated freeze-thaw and short time normal temperature transportation.

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Product Components

components	100 T
A. β-Galactoside enzyme staining fixative	100 mL
B. β-Galactosidease staining solution A	1.5 mL
C. β-Galactosidease staining solution B	1.5 mL
D. β-Galactosidease staining solution C	100 mL
E. X-Gal solution	5 mL

Note: Component E X-Gal solution should be stored in the dark.

Materials Required (Not Supplied)

1. Consumables

Centrifuge Tubes

2. Reagents

(1) 0.85% NaCl

(2) 0.7 mL isopropanol

3. Instruments

Light microscope

Experimental Procedure

1. Adherent Cell Staining (6-well plate)

(1) Cells should be plated overnight until reaching 60-80% confluence before the experiment.

(2) Remove the cell culture medium and wash with PBS once.

(3) Add 1 mL of β-galactosidase staining fixative and fix at room temperature for 10-15 minutes.

(4) Discard the β-galactosidase staining fixative and wash three times with PBS, each time for 2-3 minutes.

(5) Prepare the staining working solution according to Table 1. The total volume of the staining working solution should be calculated based on sample type and quantity.

Table 1 Staining working fluid

Reaction components	Take 1 mL of staining working fluid as an example
β-Galactosidease staining solution A	10 μL
β-Galactosidease staining solution B	10 μL
β-Galactosidease staining solution C	940 μL
X-Gal solution	40 μL

Note:

1) To minimize crystallization during sample staining, it is recommended to preheat the X-Gal solution at 37℃ ℃ for 1-3 hours before use.

2) The staining working solution should be prepared and used immediately, preferably within 15 minutes.

(6) Remove PBS and add 1 mL of staining working solution per well. Incubate in a 37℃ ℃ CO2-free incubator overnight.

Note: To prevent evaporation from affecting staining results, add water or PBS to the edge wells to reduce the "edge effect", and cover the plate with sealing film or plastic wrap.

(7) Perform observation and counting under a conventional optical microscope.

(8) Optional: Remove the staining working solution and add 2 mL of PBS for storage for several days.

2. Suspension Cell Staining

(1) Collect cells, centrifuge at 2500 × g for 10 minutes, and wash once with PBS.

(2) Add 1 mL β-galactosidase staining fixative and fix at room temperature for 10-15 minutes.

Note: Slowly shake the cells on a shaker to prevent clumping.

(3) Centrifuge at 2500 × g for 10 minutes, remove β-galactosidase staining fixative, and wash three times with PBS (1-3 minutes per wash).

(4) Prepare the staining working solution according to Table 1. The total volume should be calculated based on sample type and quantity.

(5) Centrifuge at 2500×g for 10 minutes and remove the PBS. Add 0.5-1 mL of staining working solution to each tube and incubate at 37°C for overnight.

(6) Aspirate part of the cells and transfer to a glass slide or 6-well plate for observation and counting under a standard optical microscope.

(7) Optional: Remove the staining working solution, add 1 mL of PBS, and store for several days.

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Notes

1. Before use, centrifuge the product to the tube bottom immediately before proceeding with subsequent experiments.
2. The senescence β-galactosidase detection relies on specific pH conditions. Since high CO2 concentrations in CO2 incubators can affect the pH of staining working solution, 37°C incubation should not be performed in CO2 incubators.
3. X-Gal solution may freeze when stored at-20°C ℃ or 4°C ℃. It can be fully thawed by 2-5 minutes at room temperature or in a 37°C water bath with gentle shaking. For aliquoting the solution or preparing staining working solutions, use polypropylene (PP) or glass materials instead of polystyrene (PS) containers such as cell culture plates or serum pipettes.
4. Minor sediment may appear at the bottom of tube B of β-galactosidase staining solution. Shake vigorously to ensure complete dissolution before staining.
5. This kit is theoretically applicable to frozen sections but not paraffin sections. For staining, refer to relevant literature and conduct preliminary experiments to optimize protocols.
6. β-galactosidase staining fixative contains toxic and corrosive substances. For safety, wear lab coats and disposable gloves to avoid direct contact with human skin or inhalation.
7. This product is strictly for research purposes only and must not be used for clinical diagnosis or treatment.

This product is for research use only!