Cellular Senescence Staining Kit

Cellular Senescence Staining Kit can be used to measure activity of senescence-associated beta Galactosidase in cells or tissue samples. Count the blue stained senescence cells using light microscope

Catalog number: ARG82262

Package: 50 assays

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INTRODUCTION

Cellular senescence is a phenomenon characterized by the cessation of cell division. In their experiments during the early 1960s, Leonard Hayflick and Paul Moorhead found that normal human fetal fibroblasts in culture reach a maximum of approximately 50 cell population doublings before becoming senescent. This process is known as "replicative senescence", or the Hayflick limit. Hayflick's discovery of mortal cells paved the path for the discovery and understanding of cellular aging molecular pathways. Cellular senescence can be initiated by a wide variety of stress inducing factors. These stress factors include both environmental and internal damaging events, abnormal cellular growth, oxidative stress, autophagy factors, among many other things. [Provide by Wikipedia: Cellular senescence]

Senescence-associated beta-galactosidase (SA-β-gal or SABG) is a hypothetical hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides only in senescent cells. Senescence-associated beta-galactosidase, along with $p16^{Ink4A}$, is regarded to be a biomarker of cellular senescence. [Provide by Wikipedia: Senescence-associated beta-galactosidase]

PRINCIPLE OF THE ASSAY

This Cellular Senescence Staining Kit employs a substrate to measure the cellular senescence biomarker. SA-β-galactosidase catalyzes the hydrolysis of X-gal, which produces a blue color.

MATERIALS PROVIDED & STORAGE INFORMATION

Store X-gal solution protected from light at -20°C. Store all other components at 4°C. Avoid multiple freeze / thaw cycles. Use the kit before expiration date.

Component	Quantity	Storage information
100X Fixing Solution	1.5 mL	4°C
Staining solution A	1.5 mL	4°C
Staining solution B	1.5 mL	4°C
Staining solution C	4.5 mL	4°C
Staining solution D	4.0 mL	4°C
X-gal Solution	2 X 1.5 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- 37°C Incubator
- Light microscope
- Deionized or Distilled water
- 1X PBS
- Pipettes and pipette tips

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store X-gal solution protected from light at -20°C. Store all other components at 4°C.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

All the cell type can be determine by this Cellular Senescence Assay kit. The cell culture methods can refer to the cell source, like ATCC Product Sheet.

REAGENT PREPARATION

- **1X Fixing Solution:** Prepare a 1X Fixing Solution by diluting the provided 100X stock 1:100 in 1X PBS. Store the diluted solution at room temperature for up to six months.
- **Cell Staining Working Solution:** Prepare FRESH cell staining working solution based on the number of samples. The chart below is suggested for samples in 35 mm plate, and may be modified accordingly to suit other culture plate sizes.

Reagent	1 dish (35 mm)	5 dish (35 mm)	10 dish (35 mm)
Staining Solution A	20 µL	100 µL	200 µL
Staining Solution B	20 µL	100 µL	200 µL
Staining Solution C	80 µL	400 µL	800 µL
Staining Solution D	60 µL	300 µL	600 µL
X-Gal Solution	50 µL	250 µL	500 µL
Distilled water	1.77 mL	8.85 mL	17.7 mL
Total	**2 mL**	**10 mL**	**20 mL**

ASSAY PROCEDURE

1. Aspirate the culture medium from the senescent cells expressing SA-β-gal.
2. Wash the cells twice with **3 mL** of **1X cold PBS** and aspirate the final wash.
3. Add **2 mL** of **1X Fixing Solution**. Incubate at **room temperature** for **5 minutes**.
4. Remove the 1X Fixing Solution and wash the fixed cells three times with **3 mL** of **1X PBS**.
5. Aspirate the final wash, and completely cover cells by adding **2 mL** of freshly prepared **Cell Staining Working Solution**.
6. Incubate the cells at **37°C** for **4 hours to overnight** in the dark.
7. Remove the **Cell Staining Working Solution**, then wash the stained cells **twice** with **3 mL** of **1X PBS** and store cells in **1X PBS**. For long-term storage, overlay the cells with **1X PBS containing 20% Glycerol**. Store at **4°C**.
   Note: Excess amount of salt crystals can be removed by briefly incubating the stained sample with DMSO.
8. Count the blue stained senescence cells using light microscope.