Cellular Senescence Staining Kit (Fluorometric)

Cellular Senescence Staining Kit (Fluorometric) can be used to measure activity of senescence-associated beta Galactosidase in cells or tissue samples by fluorescence microscopy or flow cytometry.

Catalog number: ARG82263

Package: 10 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 (Fluorometric) employs a fluorogenic substrate to measure the cellular senescence biomarker. SA-β-galactosidase catalyzes the hydrolysis of the galactosyl residues emits green fluorescence and remains confined within the cell.

MATERIALS PROVIDED & STORAGE INFORMATION

Aliquot and store all components at -20°C. Avoid multiple freeze / thaw cycles.

Use the kit before expiration date.

Component	Quantity	Storage information
1000X Cell Pretreated Solution	25 µL	-20°C
200X SA-β-Gal Substrate	100 µL	-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.
- Aliquot and store all components at -20°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 Cell Pretreated Solution:** Prepare desired amount of 1X Cell Pretreatment Solution by diluting the provided 1000X stock 1:1000 in cell culture medium. (E.g., add 4 µL of 1000X Cell Pretreatment Solution to 4 mL of cell culture medium) Use the diluted cell pretreatment solution within 4 hours.

ASSAY PROCEDURE

1. Aspirate the cell culture medium from the senescent cells expressing SA-β-gal.
2. Add **2 mL** of **1X Cell Pretreated Solution.** Incubate at **37°C** for **2 hours.**
3. Add **10 µL** of **200X SA-β-Gal Substrate Solution** directly to the cells in **1X Cell Pretreatment Solution.** Gently mix and incubate at **37°C** for **4 hours to overnight.**
4. Wash the stained cells **three** times with **3 mL** of **1X PBS**.
5. Analyze the senescent cells by one of the following methods
   A. Flow cytometer after cells are trypsinized and washed in cold 1X PBS containing 2% FBS.
   B. Fluorescence microscope (Excitation: 485 nm / Emission: 520 nm).