Cellular Senescence Assay Kit (Fluorometric)

Cellular Senescence Assay Kit (Fluorometric) can be used to measure activity of senescence-associated beta Galactosidase in cell lysate.

Catalog number: ARG82213

Package: 120 assay

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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.

The physiological importance for cell senescence has been attributed to prevention of carcinogenesis, and more recently, aging, development, and tissue repair. Senescent cells contribute to the aging phenotype, including frailty syndrome, sarcopenia, and aging-associated diseases. Senescent astrocytes and microglia contribute to neurodegeneration. [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 Assay Kit (Fluorometric) employs a fluorogenic substrate to measure SA-β-Gal activity, the cellular senescence biomarker, in cell lysate samples. This quantitative assay uses cell lysate for both SA-β-galactosidase activity determination and normalization of samples containing different cell numbers. Each kit provides sufficient quantities to perform up to 120 assays in a 96-well plate.

MATERIALS PROVIDED & STORAGE INFORMATION

Store SA-β-gal substrate solution protected from light at -20°C upon received. Avoid multiple freeze/thaw cycles. Store all other components at room temperature. Use the kit before expiration date.

Component	Quantity	Storage information
2X Cell Lysis Buffer	10 mL	Room temperature
2X Reaction Buffer	10 mL	Room temperature
20X SA-β-Gal Substrate	300 µL	-20°C (protect from light)
Stop Solution	25 mL	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader (excitation: 360 nm / emission: 465 nm)
- 96-well microplate
- 96-well fluorescence microplate
- 37°C Incubator
- Deionized or Distilled water
- Protease inhibitors (E.g. PMSF)
- β-mercaptoethanol
- Protein Assay Reagent
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store SA-β-gal substrate solution protected from light at -20°C. Store all other components at room temperature.
- 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 may 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 Lysis Buffer:

  a. Prepare 1X Cell Lysis Buffer by diluting the provided 2X Cell Lysis Buffer stock with equal volume of distilled water.

  b. Store the diluted solution at room temperature for up to six months.

  c. Immediately before use, add proper amount of protease inhibitors such as PMSF.

- 2X Assay Buffer:

  a. Prepare immediately before use.

  b. Add β-mercaptoethanol to 2X Reaction Buffer at a final concentration of 10 mM. (If a pure liquid of β-mercaptoethanol is used, the concentration of the pure β-mercaptoethanol is 14.3M. Add 0.7µl of pure β-mercaptoethanol in 1 ml of 2X Reaction Buffer. Please confirm the concentration of the β-mercaptoethanol you used before preparation.)

  c. Dilute 20X SA-β-Gal Substrate to 1X SA-β-Gal Substrate with 10 mM β-mercaptoethanol contained 2X Reaction Buffer from step b. (e.g. add one part of 20X SA-β-Gal Substrate into 19 parts of buffer from step b) And this is 2X Assay buffer.

  d. Use immediately, and don't store this 2X Assay Buffer.

ASSAY PROCEDURE

Samples are suggested assayed in duplicates.

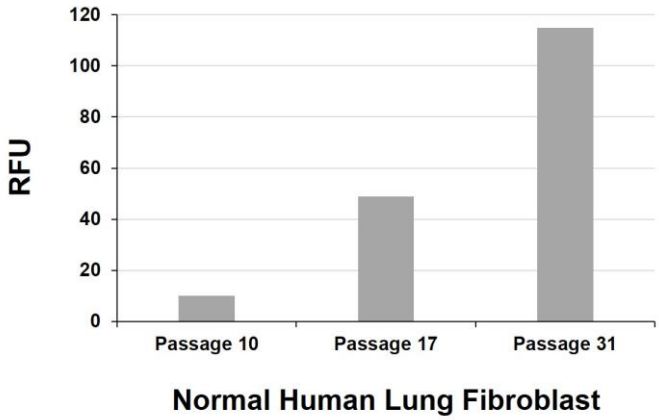
1. Aspirate the culture medium from the senescent cells.
2. Wash the cells once with **200 µL** of **cold 1X PBS** and aspirate.
3. Add **100 µL** of **cold 1X Cell Lysis Buffer** (see the table below for the required amount of 1X Cell Lysis Buffer of other plate formats).

Reagent	96-well	24-well	6-well	10 cm Dish
1X Cell Lysis Buffer	100 µL	400 µL	1000 µL	1500 µL

4. Incubate at **4°C** for **5 minutes**. Transfer the whole lysate to a microcentrifuge tube and centrifuge at 12,000 xg at **4°C** for **10 -15 minutes.** Collect supernatant as cell lysate.
5. (optional) Determine the total protein concentration of each cell lysate sample by protein assay such as Pierce's BCA protein Assay.
6. Transfer **50 µL** of the **cell lysate** to a 96-well microplate. Add **50 µL** of freshly prepared **2X Assay Buffer** in each well. Cover the plate and incubate the wells at **37°C** for **1-3 hours in the dark**.
7. Remove **50 µL** of the **reaction mixture** above to a **96-well fluorescence microplate**. Stop the reaction by adding **200 µL** of **Stop solution**.
8. Read the plate with a fluorescence microplate reader equipped for **excitation** in the **360 nm** and for **emission** in the **465 nm**.

EXAMPLE OF TYPICAL SAMPLE DATA

The following figures demonstrate typical with the 96-well Cellular Senescence Assay Kit. Fluorescence measurement was performed on a fluorescence microplate reader with a 355 nm/460 nm filter set. One should use the data below for reference only. This data should not be used to interpret actual results.



Normal Human Lung Fibroblast HFL-1 cells with different passage numbers were lysed. Lysates were allowed to incubate with SA-ß-Gal Substrate for 1hr at 37°C. SA-ß-Gal activities were measured as described in the Assay Protocol