

## Senescence Detection Kit

(Catalog #K320-250; Store kit at +4°C)

### I. Introduction:

Senescence is thought to be a tumor suppressive mechanism and an underlying cause of aging. Senescence represents an arrested state in which the cells remain viable, but not stimulated to divide by serum or passage in culture. Senescent cells display increase of cell size, senescence-associated expression of  $\beta$ -galactosidase (SA- $\beta$ -Gal) activity, and altered patterns of gene expression. The Senescence detection kit is designed to histochemically detect SA- $\beta$ -Gal activity in cultured cells and tissue sections, a known characteristic of senescent cells. The SA- $\beta$ -Gal is present only in senescent cells and is not found in presenescent, quiescent or immortal cells.

### II. Kit Contents:

Components	Volume	Color Code
Fixative Solution (1X)	125 ml	NM Bottle
X-Gal (150 mg, lyophilized)	1 vial	Green Cap
Staining Solution (1X)	125 ml	WM Bottle
Staining Supplement (100X)	1.5 ml	Red Cap

### III. Senescence Detection Protocol:

#### A. General Consideration & Reagent Preparations:

- The following protocol is designed for each well in a 12-well plate. For using large plate, increase the volume proportionally (e.g., For 6-well plate, double the volume).
- Prepare 1X PBS Solution (not provided). Prepare 3 ml per well.
- Prepare X-gal Solution: Weigh 20 mg X-gal, dissolve in 1 ml DMSO or DMF (N-N-dimethylformamide, not provided) to prepare a 20X stock solution. Excess X-gal solution can be stored at  $-20^{\circ}\text{C}$  (protect from light) for one month. Always use polypropylene container or glass to make and store X-gal. Do not use polystyrene.
- Staining Solution and Staining Supplement: If precipitation occurs, simply warming up the solution to solublize the precipitates.

#### C. Staining Protocol:

- Remove culture medium and wash cells once with 1 ml of 1X PBS.
- Fix the cells or frozen tissue sections with 0.5 ml of Fixative Solution for 10-15 minutes at room temperature.
- While the cells in the Fixative Solution, prepare the Staining Solution Mix. Using polypropylene plastic tube only. Prepare enough solution for the number of wells to be stained. For each well, prepare
  - 470  $\mu\text{l}$  of Staining Solution
  - 5  $\mu\text{l}$  of Staining Supplement
  - 25  $\mu\text{l}$  of 20 mg/ml X-gal in DMF
- Wash the cells two times with 1 ml of 1X PBS.
- Add 0.5 ml of the Staining Solution Mix to each well. Cover the plate. Incubate overnight at  $37^{\circ}\text{C}$ .

6. Observe cells under a microscope for development of blue color (200X total magnification).

7. For long-term storage of the stained plates, remove the Staining Solution and overlay the cells with 70% glycerol. Store at  $4^{\circ}\text{C}$ .

### IV. Storage and Stability:

- Store kit at  $4^{\circ}\text{C}$  or  $-20^{\circ}\text{C}$ , protected from light. Store reconstituted X-gal in  $-20^{\circ}\text{C}$ . All components supplied are stable for 1 year.

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- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and siRNA Vectors

#### Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

#### Cell Proliferation & Senescence

- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

#### Cell Damage & Repair

- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

#### Signal Transduction

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- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

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- CETP & PLTP Activity Assay & Drug Discovery Kits
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