# Senescence β-Galactosidase Staining Kit

Cat #: D-AKE3030

Size: 100T

Storage: Stored at -20°C for 12 months

## Assay Principle

Cells are the basic unit of biological structure and function, as well as the basic unit of biological aging. Cellular senescence is morphologically manifested as degeneration of cell structure, such as nuclear membrane depression, which eventually leads to nuclear membrane collapse, chromatin structure changes, and the number of hyperdiploid and abnormal polyploid cells increases; cell membrane fragility increases. Sexual permeability decreases, and the types and numbers of membrane receptors and the sensitivity to ligands change; lipofuscin accumulates in cells, and many organelles and intracellular structures undergo degeneration. Cell senescence is physiologically manifested as functional decline and low metabolism, such as cell cycle arrest, loss of cell replication ability, weakened responsiveness to mitogenic stimulation, and changes in responsiveness to pro-apoptotic factors; intracellular enzyme active centers are oxidized, Enzyme activity decreased, protein synthesis decreased, etc. The senescent cells can no longer replicate, but they still maintain metabolically active and stain positive for senescence-associated beta-galactosidase activity, which is considered to be a biomarker of cellular senescence. Senescence β-Galactosidase Staining Kit is a kit for staining and detecting senescent cells or tissues based on the up-regulation of senescence-related β-galactosidase activity level during aging. With X-Gal as the substrate, under the condition of pH 6.0, the senescent β-galactosidase catalyzes the formation of dark blue products. So that cells or tissues expressing β-galactosidase that turned blue were observed under an optical microscope. This kit is suitable for the detection of senescence in cultured cells and tissue sections. It only stains senescent cells, and does not stain pre-senescence cells (senescent cells), quiescent cells (static cells), immortal cells (immortal cells) or tumor cells.

## Materials Supplied and Storage Conditions

| Kit components | Size（100T） | Storage conditions |
|---|---|---|
| 10×Fixation Buffer | 15 mL | -20°C |
| 10×PBS | 50 mL | 4°C |
| Reagent A | 1.5 mL | -20°C |
| Reagent B | 1.5 mL | -20°C |
| Reagent C | 1.5 mL | -20°C |
| X-Gal solution | 6 mL | -20°C, protected from light |

## Materials Required but Not Supplied

·Optical microscope

·37°C incubator without $CO_2$

·Pipette and pipette tip, polypropylene tube (15 or 50 mL)

·Various glassware for preparing reagents and buffer solutions

·Deionized water, glycerin

·Sealing film, 6-well plate

## Reagent Preparation

**1×PBS:** Before use, dilute with deionized water to 1×PBS. Equilibrate to room temperature before use. Store at 4°C.

**1×Fixation Buffer:** Before use, dilute with 1×PBS to 1×Fixation Buffer. Equilibrate to room temperature before use. Store at -20°C.

**Reagent A:** Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

**Reagent B:** Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

**Reagent C:** Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

**X-Gal solution:** Ready to use as supplied. Equilibrate to 37°C before use. Store at -20°C, protected from light. **Warm the X-gal Solution at 37°C for 1 hour is very important to avoid formation of aggregates that could interfere with the visualization of the stained cells.**

**Staining Working Solution:** Mix the reagents in the ratio of 10 µL Reagent A, 10 µL Reagent B, 10 µL Reagent C, 50 µL X-gal solution and 920 µL 1×PBS, and then adjust to pH 6.0 with NaOH or HCl.

**Note: Use containers or glass containers of polypropylene materials when preparing the Staining Working Solution. A small amount of flocculent precipitate may appear, it will be completely dissolved after shaking and mixing. Be ensured to be completely dissolved before use.**

## Assay Procedure

**Note: Staining Working Solution is toxic and corrosive to the human body. Please be careful when handling and pay attention to effective protection. Avoid contact with human body or direct inhalation.**

**I-A For adherent cells:**

1. Cultivate the cells directly on the cover glass of the 6-well plate and perform the required treatment.
2. Aspirate the cell culture solution and wash twice with 1×PBS.
3. Add 1 mL 1×Fixation Buffer to each well and fix for 15 min at room temperature. For other types of culture plates, the amount of Fixation Buffer and subsequent solutions should be operated with reference to this ratio.
4. Aspirate the Fixation Buffer, and wash the cells 3 times with 1×PBS.
5. Aspirate 1×PBS and add 1 mL Staining Working Solution to each well.
6. Incubate overnight in a 37°C incubator without $CO_2$ until the cells are stained blue.

**Note: Seal the 6-well plate with parafilm to prevent the cells from drying out, and choose an appropriate staining time. Since cell senescence staining is related to pH, it is impossible to culture cells in a 37°C incubator without $CO_2$.**

7. Place the cells under an optical microscope for observation. Count the number of blue cells and total cells, and calculate the percentage of cells expressing β-galactosidase (senescent cells). After staining, if you cannot observe and count in time, you can remove the Staining Working Solution, add 2 mL 1×PBS, and store for several days at 4°C or cover the cells with 70% glycerol solution for long-term storage at 4°C.

**I-B For suspension cells:**

1. Centrifuge at 1,000 rpm for 5 min to collect the cells in a 1.5 mL centrifuge tube, and wash twice with 1×PBS.

2. Add 1 mL 1×Fixation Buffer to each tube, and fix for 15 min at room temperature. During fixation, you can slowly shake it on a shaker to prevent the cells from forming clumps.

3. After centrifugation at 1,000 rpm for 5 min, aspirate the Fixation Buffer, and wash the cells 3 times with 1×PBS.

4. Aspirate 1×PBS and add 1 mL Staining Working Solution to each tube.

5. Incubate overnight in a 37°C incubator without $CO_2$ until the cells are stained blue.

6. Take part of the stained cells, drop them onto a glass slide or a 6-well plate, and observe under an ordinary optical microscope. Count the number of blue cells and total cells, and calculate the percentage of cells expressing β-galactosidase (senescent cells). If you cannot observe the count in time, you can remove the Staining Working Solution, add 2 mL 1×PBS, and store for several days at 4°C or cover the cells with a 70% glycerol solution for long-term storage at 4°C.

**I-C For tissue sections:**

For paraffin sections, perform deparaffinization and hydration according to conventional methods. Follow the steps below directly for frozen sections.

1. For the prepared tissue section, add an appropriate volume of 1×Fixation Buffer to cover the tissue adequately, and fix for 15 min at room temperature.

2. Aspirate the Fixation Buffer and wash the cells 3 times with 1×PBS.

3. Aspirate 1×PBS and add an appropriate amount of Staining Working Solution.

4. Incubate overnight in a 37°C incubator without $CO_2$ until the cells are stained blue.

5. Place the tissue section under an optical microscope for observation. Count the number of blue cells and total cells, and calculate the percentage of cells expressing β-galactosidase (senescent cells). After staining, if it cannot be observed in time, cover the tissue section with 70% glycerol solution for long-term storage at 4°C.

## Precautions

1. This kit only stains senescent cells, not presenescent cells, quiescent cells, immortal cells or tumor cells.

2. X-Gal solution will freeze when stored at -20°C or 4°C, and it can be completely dissolved in a water bath at room temperature or 37°C for 2-5 min with proper shaking.

3. Cell senescence β-galactosidase staining reaction depends on specific pH conditions, and the staining reaction cannot be performed in a $CO_2$ incubator. The higher concentration of $CO_2$ in the incubator will affect the pH value of the Staining Working Solution and cause staining failure. For the porous plate, it can be sealed with parafilm or plastic wrap to prevent evaporation.

4. If there is precipitation after the reagent is thawed or before use, it must be ensured that all the precipitation is dissolved before use. When Reagent C is just taken out of the kit, there may be a small amount of precipitation at the bottom of the tube, which is a normal phenomenon. After mixing thoroughly, the precipitation will be completely dissolved. Make sure to use it after all of it is dissolved. When preparing the Staining Working Solution, a small amount of flocculent precipitate may also appear, which will be completely dissolved after shaking and mixing, and it must be used before it is completely dissolved.

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.