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## **Senescence-Associated $\beta$ -Galactosidase (SA- $\beta$ -Gal) Stain Kit**

GI580 (100 test)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Introduction

The Senescence  $\beta$ -Galactosidase Staining Kit is a staining kit for senile cells or tissues based on the up-regulation of SA- $\beta$ -Gal (senescence-associated  $\beta$ -galactosidase) activity during aging. Aging of cells or tissues can be observed under optical microscopes. Cell aging is also considered as a way for organisms to inhibit tumors and a potential cause of organism aging. The kit can be used for senescence detection of cultured cells and tissue sections.

Using X-Gal as substrate, the Senescence  $\beta$ -Galactosidase Staining Kit produces dark blue products catalyzed by aging-specific  $\beta$ -galactosidase. Cells or tissues expressing  $\beta$ -galactosidase that turn blue are easily observed under optical microscopy. This kit only stains senescent cells. For tissue sections or blocks, the number of samples that can be detected depends on the size of the sample. For ordinary sections or 6-well plate, at least 100 samples can be detected.

## Kit Components

Reagent	100T	Storage
Reagent(A): $\beta$ -Gal Fixative	100mL	2-8°C, avoid light
Reagent(B): X-Gal Solution	5mL	-20°C, avoid light
Reagent(C): $\beta$ -Gal Stain Solution A	1mL	2-8°C, avoid light
Reagent(D): $\beta$ -Gal Stain Solution B	1mL	2-8°C, avoid light
Reagent(E): $\beta$ -Gal Stain Solution C	100mL	2-8°C
Mix Reagent B, C, D, E, in proportion of 5:1:1:93 as Dyeing Working Solution. It is ready to use		

**Storage:** -20°C, avoid light, valid for 1 year

## Protocol

Note:

- It is recommended to use polypropylene (PP) container to prepare dyeing working solution, polystyrene (PS) container should be used to prepare dyeing working solution.
- The incubation at 37°C during dyeing should not be carried out in carbon dioxide incubator.

### Adherent Cells Stain

1. For cells cultured in 6-well plates, remove the cell culture medium and wash once with PBS or HBSS. Add 1mL  $\beta$ -Gal Fixative to each well and fix at room temperature for 15 minutes. For other types of culture plates, the amount of fixative solution and subsequent solution has to be calculated according to this ratio.
2. Remove  $\beta$ -Gal Fixative and wash cells 3 times with 1X PBS for 3 minutes each time.
3. Remove washing solution and add 1mL Dyeing Working Solution to each well.
4. Incubate for 12-24hours at 37°C; the 6-well plate can be sealed with parafilm to prevent evaporation.
5. View under the optical microscope. If you can't view and count in time, you can remove the Dyeing Working Solution, add 2mL PBS, and store it at 2-8°C for seven days.

### Suspension Cell Stain

1. Collect the cells into 1.5mL eppendorf tube by centrifugation, wash once with PBS or HBSS, and fix at room temperature for 15 minutes with 1mL  $\beta$ -Gal Fixative. Slowly shake the tubes to avoid formation of cell clumps.
2. Centrifuge and remove  $\beta$ -Gal Fixative, wash cells with 1X PBS 3 times for 3 minutes each time.
3. Centrifuge and remove the washing solution then add 0.5-1mL Dyeing Working Solution to each tube. Incubate for 12-24hours at 37°C.

4. Transfer a small volume to a glass slide and view under optical microscope. If cells are to be stored, centrifuge and remove the dyeing working solution then adding 1mL PBS at 2-8°C can keep for seven days. After centrifugation, the cells can also be made into a smear, and sealed with water based sealing agent. In this way it can be stored for a long time at 2-8°C.

### **Tissue Sections Stain**

1. For enzymatically protected frozen slices, follow the steps below. (See Note 3)
2. Add a suitable volume of  $\beta$ -Gal Fixative to cover the tissue adequately and fix it for more than 15 minutes.
3. Wash the tissues 3 times with 1X PBS for more than 5 minutes each.
4. Remove washing solution.
5. Incubate for 12-24 hours at 37°C.
  - a. For drip dyeing: the tissue sections can be placed in the wet-box to prevent evaporation
  - b. For soak dyeing: immerse the sections in dye container in a closed condition.
6. View under optical microscope. To store the sections, seal with glycerol gelatin.

### **Note**

1. The  $\beta$ -Gal Fixative has certain corrosiveness and toxicity. Use protective laboratory wears while handling the reagents.
2. The  $\beta$ -galactosidase staining reaction depends on specific pH conditions and cannot be performed in a carbon dioxide incubator. The high concentration of carbon dioxide in the carbon dioxide incubator used for cell culture can affect the pH value of the staining solution, leading to staining failure.
3. The stability of galactosidase is poor, and it is easily inactivated when paraformaldehyde is used to fix or prepare paraffin sections. It is recommended to prepare quick-frozen sections, or use enzyme protection special fixing solution for fixing and freezing sections. Tissue immersion in galactosidase fixative stabilizes enzyme activity for at least one week.
4. Polypropylene containers or glass containers should be used in the preparation of dyeing working solution instead of polystyrene containers. But dyeing can be carried out in polystyrene containers, such as 6-well plate.
5. If the smear needs to be stored for a long time, it can be washed twice with distilled water after dyeing, and the neutral gum seal can be made.