# Cell Senescence β-Galactosidase Staining Kit

## Introduction

Cell Senescence β-Galactosidase Staining Kit is a kit for the detection of senescence-specific β-galactosidase (SA-β-Gal) in cells or frozen sections using X-gal as substrate. When cells sense, they express a special β-galactosidase, SA-β-Gal, which has high enzymatic activity at pH 6.0. At the right pH, SA-β-Gal can catalyze X-gal to produce the blue product. This product can be observed by ordinary light microscopy, and its content is positively correlated with the level of cellular senescence.

This kit stains only senescent cells, not presenescent cells, quiescent cells, immortalized cells, or tumor cells. This kit is optimized to be compatible with polystyrene consumables or containers such as common cell culture plates, pipettes, etc., which can effectively avoid poor staining results caused by incompatible consumables or containers. In addition, the working solution prepared by this kit will not produce precipitation during use, which is more convenient.

## Components and Storage

| Components | K2185-100 T |
|---|---|
| Fixative Solution | 100 mL |
| X-gal Solution | 5 mL |
| Staining Solution A | 1 mL |
| Staining Solution B | 1 mL |
| Staining Solution C | 100 mL |
| Store the kit at -20°C, stable for 1 year. X-gal Solution should be stored at -20°C away from light. | |

## Protocol

1. **For cell samples:**

   1) **Cell fixation:** For 6-well plates, remove the medium, wash 1 time with PBS, then add 1 mL of Fixative Solution per well and fix for 15 min at room temperature. The Fixative Solution for other plates can be adjusted according to the size of the plate.

   2) **Wash:** Remove the Fixative Solution and wash each well with PBS 3 times for 3 min each.

   3) **Preparation of staining solution:** For 6-well plates, refer to the table below to prepare the working

solution, and the amount of staining solution for other plates can be adjusted according to the size of the plate.

| | |
|---|---|
| X-gal Solution | 50 μL |
| Staining Solution A | 10 μL |
| Staining Solution B | 10 μL |
| Staining Solution C | 930 μL |

4) **Staining:** Remove PBS and add 1 mL of staining solution per well. Incubate overnight at 37°C and seal the 6-well plate with parafilm or plastic wrap to minimize liquid evaporation.

***Note:** Incubation should not be performed in a cell $CO_2$ incubator, as the high concentration of $CO_2$ in the incubator can affect the pH of the staining solution, resulting in staining failure.

5) **Detection:** Observation under a microscope. If it is not possible to observe in time, remove the staining solution, add an appropriate amount of PBS and then the sample can be kept at 4°C for several days. Or the sample can be stored for a longer time at 4°C after mounting.

***Note:** After adding the staining solution, due to the evaporation of the solution or other reasons, crystals may appear and affect the imaging. At this time, the staining solution can be removed after the staining is completed, wash cells with an appropriate amount of 70% ethanol, and after the crystallization is dissolved, the 70% ethanol can be replaced with PBS or normal saline before taking pictures. 70% ethanol washing does not affect staining results.

2. **For Frozen sections (not recommended for paraffin sections):**

1) **Pretreatment:** Warm frozen sections to room temperature. Wash the sections 3 times with PBS for no less than 5 min each.

2) **Fixation:** Place the sections in a 6-well plate, add an appropriate amount of Fixative Solution, and fix at room temperature for no less than 15 minutes.

3) **Washing:** Remove the Fixative Solution and wash the sections 3 times with PBS for no less than 5 min each.

4) **Staining solution preparation:** For 6-well plates, refer to the table below to prepare the working solution.

| | |
|---|---|
| X-gal Solution | 50 μL |
| Staining Solution A | 10 μL |
| Staining Solution B | 10 μL |
| Staining Solution C | 930 μL |

5) **Staining:** Remove PBS and add 1 mL of staining solution per well. Incubate overnight at 37°C and seal

the 6-well plate with parafilm or plastic wrap to minimize liquid evaporation.

***Note:** Incubation should not be performed in a cell $CO_2$ incubator, as the high concentration of $CO_2$ in the incubator can affect the pH of the staining solution, resulting in staining failure.

6) **Detection:** Observation under a microscope. If it is not possible to observe in time, remove the staining solution, add an appropriate amount of mount solution and then the sample can be kept at 4°C for a longer time.

***Note:** After adding the staining solution, due to the evaporation of the solution or other reasons, crystals may appear and affect the imaging. At this time, the staining solution can be removed after the staining is completed, wash cells with an appropriate amount of 70% ethanol, and after the crystallization is dissolved, the 70% ethanol can be replaced with PBS or normal saline before taking pictures. 70% ethanol washing does not affect staining results.

## Note

1. The Fixative Solution in this kit is toxic and corrosive to the human body, please pay attention to the precautions when handling to avoid direct contact with the human body.
2. X-gal freezes when stored at -20°C. Thaws it completely in a few minutes at room temperature or in a 37°C water bath.
3. Precipitation may be observed in Staining Solution B in this kit after thawing, and the pellet will be completely dissolved after thorough mixing or proper vortexing.
4. Incubation should not be performed in a cell $CO_2$ incubator, as the high concentration of $CO_2$ in the incubator can affect the pH of the staining solution, resulting in staining failure.
5. In paraffin embedding, temperature and fixing solution may lead to inactivation of β-Galactosidases, resulting in staining failure. Therefore, this kit is not recommended for paraffin section staining. If must be used in the paraffin section, it is recommended to explore the best conditions.
6. When using multi-well plates, such as 96-well plates, the edge wells of the 96-well plate are prone to liquid evaporation issues if overnight incubation is required. It is recommended to discard the circumference of the 96-well plate and add sterile water, medium, or PBS instead, and place the 96-well plate in the incubator near the water source.
7. For your safety and health, please wear lab coats and gloves during the experiment.
8. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com