# Lysosomal β-Galactosidase Staining Kit

## Introduction

Lysosomal β-Galactosidase Staining Kit is a kit for the detection of lysosomal acidic β-galactosidase in cell or tissue sections using X-gal as substrate, which is often used as a control for cell senescence detection. High levels of lysosomal acidic β-galactosidase can be detected in most normal cells, while β-galactosidase, which is specific for cellular senescence, is only expressed during senescence, so this kit can be used as a control stain for senescent β-galactosidase. The X-gal provided in this kit can produce a blue product catalyzed by lysosomal acidic β-galactosidase, and the expression of lysosomal acidic β-galactosidase can be detected by microscopy.

This kit is only used for staining lysosomal acidic β-galactosidase, and cannot stain senescence-specific β-galactosidase or exogenously transferred E. coli β-galactosidase. 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 cannot produce precipitation during use, which is more convenient.

## Components and Storage

| Components | K2181-100 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.

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.

2. **For tissue sections:**

1) **Pretreatment:** Paraffin sections need to be deparaffinized and hydrated as usual. Frozen sections can skip this step directly.

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.

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

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. 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.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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