β-Galactosidase in Situ Staining Kit

Introduction

β-Galactosidase in Situ Staining Kit is a kit for the detection of β-galactosidase in cells or tissues using X-gal as substrate. The principle of detection is that X-gal can produce a blue product catalyzed by β-galactosidase, and the expression of β-galactosidase can be detected by ordinary light microscopy.

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	K2182-100 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 transfection:** Transfect Plasmids expressing β-galactosidase into cells by appropriate methods, and detect after transfection 24-48 h.

   2) **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 10 min at room temperature. The Fixative Solution for other plates can be adjusted according to the size of the plate.

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

   4) **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

5) **Staining:** Remove PBS and add 1 mL of staining solution per well. Incubate at 37°C for 0.2-2 h (or longer) until some cells are blue 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 PBS and then the sample can be kept at 4°C for several days. If transfection efficiency needs to be calculated, transfection efficiency = number of staining positive cells/total number of cells x 100%.

2. **For tissue sections or tissue blocks of very small volumes:**

1) **Fixation:** Place tissue sections or tissue blocks in a 6-well plate, add an appropriate amount of Fixative Solution, and fix at room temperature for no less than 10 minutes. It is recommended to extend the fixation time of tissue blocks appropriately.

2) **Wash:** Remove the Fixative Solution and wash the tissue 3 times with PBS for no less than 5 min each. It is recommended to extend the washing time of tissue blocks appropriately.

3) **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

4) **Staining:** Remove PBS and add 1 mL of staining solution per well. Incubate at 37°C for 0.2-2 h, or longer. If the incubation time is long, the 6-well plate can be sealed with parafilm or plastic wrap to minimize liquid evaporation.

5) **Washing:** Remove the staining solution and wash the tissue with PBS 3 times for no less than 5 min each time. It is recommended to extend the washing time of tissue blocks appropriately.

6) **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.

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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