β-Galactosidase Staining Kit

Catalog No. 35001

(version B)

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Overview

The β-galactosidase Staining Kit provides an easy-to-use and efficient method to determine the percentage of cells expressing *lac*Z following transient or stable transfection. The gene product of *lac*Z, β-galactosidase, catalyzes the hydrolysis of X-gal, which produces a blue color that is easily visualized.

This kit can also be used to determine the efficiency of direct delivery of β-galactosidase protein by Chariot™ Protein Delivery Reagent (Catalog Nos. 30025 & 30100).

product	format	catalog no.
β-Galactosidase Staining Kit	75 rxns	35001

Kit Components and Storage

β-Galactosidase Staining Kits are for research use only. Not for use in diagnostic procedures. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Composition	Storage
Staining Solution 1	1.5 ml	400 mM Potassium ferrocyanide	-20°C
Staining Solution 2	1.5 ml	400 mM Potassium ferricyanide	-20°C
Staining Solution 3	1.5 ml	200 mM Magnesium chloride	-20°C
10X PBS	100 ml	0.17 M KH_2PO_4 0.05 M Na_2HPO_4 1.5 M NaCl	RT or -20°C
10X Fixing Solution	17 ml	7% Formaldehyde 0.5% Glutaraldehyde in 10X PBS	-20°C
X-gal	8 ml	20 mg/ml X-gal in DMF	-20°C

Additional materials required

- Polypropylene or glass tube for Complete Staining Solution
- 37°C incubator
- Light microscope

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Warning: The 10X Fixing Solution contains formaldehyde and glutaraldehyde. Formaldehyde is poisonous and can be absorbed through the skin. Glutaraldehyde is corrosive and is a carcinogen that can also be absorbed through the skin. Potassium ferricyanide and potassium ferrocyanide are harmful if swallowed, contacted with skin or inhaled.

Buffer Preparation

Dilute the 10X Fixing Solution and the 10X PBS in sterile H_2O to a 1X final concentration.

Prepare fresh Complete Staining Solution using the chart below based on the number of samples to be processed. Be sure to use a polypropylene plastic or glass tube.

Complete Staining Solution Preparation Chart

Note: Complete Staining Solution must be made fresh for each experiment.

Reagents	1 Plate (35 mm)	5 Plates (35 mm)	10 Plates (35 mm)
Staining Solution 1	20 µl	100 µl	200 µl
Staining Solution 2	20 µl	100 µl	200 µl
Staining Solution 3	20 µl	100 µl	200 µl
1X PBS	1.84 ml	9.20 ml	18.4 ml
X-gal	100 µl	500 µl	1 ml
Total	**2 ml**	**10 ml**	**20 ml**

Staining Protocol for a 35 mm Plate

1. Aspirate the medium from the cells.
2. Wash the cells with 3 ml 1X PBS.
3. Add 2 ml 1X Fixing Solution to each well. Incubate at room temperature for 5-10 minutes.
4. Wash the fixed cells twice with 2 ml 1X PBS.
5. Add 2 ml Complete Staining Solution to each well.
6. Incubate the cells at 37°C for 30 minutes to 2 hours, or as long as necessary to see blue staining of the cells.
7. Check the cells under a microscope.
8. Calculate the percentage of cells transfected with β-galactosidase.
9. For long-term storage of the plates, remove the Complete Staining Solution and overlay the cells with 70% glycerol in PBS. Store at 4°C.

Appendix

Section A: Troubleshooting Guide

Problem/question	Possible Cause	Recommendation
Cells do not stain blue	X-gal was not added to the Complete Staining Solution.	Add X-gal to the plate(s).
	Cells were not fixed correctly.	Fix cells for 10 minutes.
	Unsuccessful transfection of DNA*.	Check transfection protocol and repeat transfection using a control vector.
	Construct is not expressing β-galactosidase*.	Sequence the *lac*Z gene of your construct to make sure it is in frame with an ATG.
	Unsuccessful delivery of β-galactosidase protein (when using Chariot™ Protein Delivery Reagent).	Make sure β-galactosidase protein is still active. Consult the Troubleshooting Guide of the Chariot manual.
	Improper washing of cells after fixative treatment.	Glutaraldehyde will interfere with color development. Wash cells thoroughly.
All cells turned blue	Too much DNA was transfected*	Use less DNA in subsequent transfections.
	Your cell line may be expressing endogenous β-galactosidase.	Test this cell line using cells that have not had β-galactosidase transfected/delivered.

Section B: Calculation of Efficiency

To calculate the percentage of cells expressing β-galactosidase, use the calculation below. This calculation is also used for calculating successful delivery of the β-galactosidase protein using the Chariot Protein Delivery Reagent.

$$\frac{\text{Total number of blue cells}}{\text{Total number of cells}} \times 100 = \%\ \text{transfection}$$

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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