PRODUCT INFORMATION & MANUAL

β-Gal Staining Kit

NBP2-29546

For the Detection of Beta-Galactosidase (β-Gal) Enzyme in Transfected Cells.

For research use only. Not for diagnostic or therapeutic procedures.

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TABLE OF CONTENTS

I. INTRODUCTION

The LacZ gene encodes for the Beta-Galactosidase (β-Gal) enzyme that catalyzes the hydrolysis of β-galactosides, including lactose. The LacZ gene is among the most widely used and published reporter genes due in large part to the extreme resistance of the β-Gal enzyme to proteolytic degradation. β-Gal enzyme activity is also readily detected in stably or transiently transfected cells or transgenic tissues using a variety of substrates.

Novus' β-Gal Staining Kit is based on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), a noninducing chromogenic substrate for β-Gal. When β-Gal cleaves the glycosidic linkage in X-Gal, a soluble, colorless indoxyl monomer is produced. Subsequently, two of the liberated indoxyl moieties form a dimer which is nonenzymatically oxidized. The dimerizatation and oxidation reactions require transfer of an electron which is facilited, in this case, by ferric and ferrous ions. The resultant halogenated indigo is a very stable and insoluble dark blue compound (Fig 1). Following transfection with plasmids that contain LacZ, and the addition of X-Gal, the transfection efficiency can be determined by counting the number of blue cells in the total cell population (Fig 2).

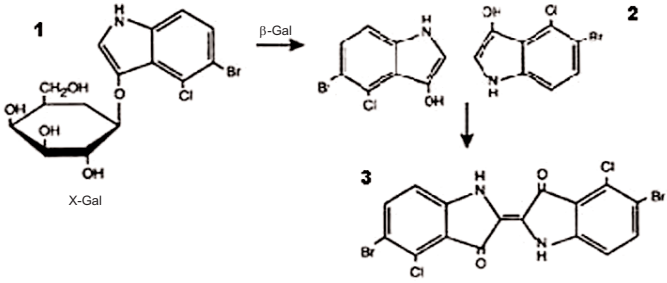

Figure 1: 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) Chromogeneic substrate. β-Gal cleavage of the glycosidic linkage in X-Gal (1) produces a colorless indoxyl monmer, 5-bromo-4-chloro-indolyl (2). Nonenzymatic oxidation and dimerization occur in the presence of ferric and ferrous ions resulting in 5,5'-di-bromo-44'-dichloro-indigo, a stable and insoluble dark blue halogenated indigocompound (3).

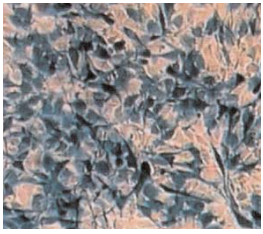

Figure 2. β-Gal activity of retroviral mediated expression of the LacZ gene. 293-10A1 cells were co-transfected with 5 μg each of pCLMFG-LacZ and pCL-Eco plasmids. Two days post transfection, 10 μl of supernatant was used to infect NIH-3T3 cells grown on chamber slides. β-Gal activity was demonstrated according to the protocol described in *Section VI. β-Gal staining Kit Procedure.*

III. KIT DESCRIPTION AND ADVANTAGES

Novus' β-Gal Staining Kit contains the necessary reagents to perform 100 assays in a 6-well plate format, providing an easy and rapid method to determine transfection efficiency by indicating β-Gal activity in individual intact cells. The transfected cells can be stained in tissue culture dishes without having to remove or manipulate the cells.[1]

Using the β-Gal Staining Kit and protocol provided, cells transfected with the bacterial LacZ gene are incubated with Fixative Solution containing 25% Glutaraldehyde. The cells are then incubated with Complete X-Gal Staining Solution containing potassium ferricyanide and potassium ferrocyanide. β-Gal cleaves X-Gal to produce a blue color in the transfected cells. The transfection efficiency is determined by counting stained and unstained cells under a microscope and calculating the percentage of stained cells in the total population. Untransfected cells should also be assayed to determine baseline levels of endogonously expressed β-Gal.

II. KIT COMPONENTS AND STORAGE

Kit Components	Quantity	Storage
Fixative Solution (25% Glutaraldehyde)	1 ml	-20ºC
X-Gal (40 mg/ml in DMSO)	600 µl	-20ºC
Staining Solution A (0.1M potassium ferricyanide)	10 ml	RT
Staining Solution B (0.1M potassium ferrocyanide)	10 ml	RT
Staining Solution C (1M MgCl2)	1 ml	RT

Caution: *Glutarldehyde is a carcinogen and may cause allergic reaction. Use it in a fume hood and discard waste as required by your institution. Avoid contact and inhalation of cyanide. Discard waste as required by your institution.*

Additional items required (not included in the kit)

- 6-Well Tissue Culture Plates *(Or tissue culture plates of choice)*
- Cells transfected with an expression plasmid encoding the LacZ gene for β-Gal expression.
- Untransfected Cells for a background control.
- Pipettor
- 37ºC incubator
- Light Microscope
- 1X PBS (approximately 10 ml per well for the 6-well format)

Plate size	Diameter	Fixative Solution	Complete X-Gal Staining Solution
60 mm plate	60 mm	5.0 ml / plate	0.5 ml / plate
100 mm plate	100 mm	7.5 ml / plate	1.0 ml / plate
150 mm plate	150 mm	10.0 ml / plate	1.5 ml / plate
96-well plate	6.4 mm	0.5 ml / well	0.05 ml / well
48-well plate	10 mm	0.75 ml / well	0.10 ml / well
24-well plate	15 mm	1.0 ml / well	0.15 ml / well
12-well plate	22 mm	1.5 ml / well	0.18 ml / well
6-well plate	35 mm	2.0 ml / well	0.25 ml / well

Table 1. Volumes recommended for Fixative Solution and Complete X-Gal Solution in tissue culture plates. Section VI β-Gal Staining Kit Procedure is opimized for use with a 6-well plate.

V. PREPARATION OF REAGENTS

Fixative Solution (0.05%Glutaraldehyde)

Fixative Solution is supplied as 25% Glutaraldehyde. Store frozen at -20ºC. It can be frozen and thawed several times. Immediately before use, thaw and dilute the required amount 500-fold in PBS. The amounts for one 35 mm diameter (6-well) plate follow:

Fixative Solution (25%Glutaraldehyde)	4.0 µl
1 X PBS	1996.0 µl
Total	2000 µl

Complete X- Gal Staining Solution

Prepare fresh Complete X-Gal Staining Solution just prior to use by combining Staining Solution A to a final concentration of 35 mM, Staining Solution B to a final concentration of 35 mM and Staing Solution C to a final consentration of 2 mM in PBS. Lastly add X-Gal to a final consentration of 1 mg/ml. The amounts for one 35 mm diameter (6-well) plate follow:

Staining Solution A	90.0 µl
Staining Solution B	90.0 µl
Staining Solution C	0.5 µl
X-Gal	6.0 µl
1 X PBS	63.5 µl
Total	250 µl

Note: *The amount of ferric (Staining Solution A) or ferrous (Staining Solution B) cyanide to use may need to be determined empirically. Higher amount causes precipitation of indole to occur more rapidly thus reducing diffusion.*

VI. β-GAL STAINING KIT PROCEDURE

This protocol is optimized for performing assays in a 6-well (35 mm) format. If you are using a different format you will need to adjust the protocol (Table 1). The protocol assumes cells were transfected with an expression plasmid encoding the LacZ gene for β-Gal expression. An untransfected cell population should be assayed in tandem to determine a baseline for endogenously expressed β-Gal.

1. Aspirate tissue culture medium from cells 24-72 h post-transfection.
2. Add 2 ml Fixative Solution (0.05% Glutaraldehyde in PBS) to each well and incubate at RT for 5 to 15 min.

   Note: *Longer incubation may inhibit enzyme activity in subsequent steps.*
3. Prepare Complete X-Gal Staining Solution.
4. Discard Fixative Solution using proper chemical disposal protocol for your institution.
5. Wash cells 3X in 2 ml 1X PBS at RT as follows:

   - Rinse with 2ml 1X PBS and aspirate off.
   - Add 2ml 1X PBS and incubate at RT for 10 min. Aspirate off.
   - Rinse with 2 ml 1X PBS and aspirate off.

   Note: *Thorough washing is important to remonve the Fixative Solution to prevent inhibition of the enzyme reaction.*
6. Add 250 µl Complete X-Gal Staining Solution to each well (just enough to cover the cells).
7. Incubate 1 hr to overnight at 37°C.
8. Aspirate off the Complete X-Gal Staining Solution and rinse with 2 ml 1X PBS.
9. Add 2 ml 1X PBS and proceed to examination of cells.

   Note: *Cells expressing the LacZ gene will test positive for β-Gal and will stain blue in the presence of X-Gal. Blue color seen by naked eye is an indication of strong staining.*
10. Examine cells using light microscopy, counting the number of stained cells versus unstained cells in randomly selected fields.
11. Calculate the percentage of stained cells in the total population:

$$\frac{\text{Blue Cells}}{\text{Total Cells}} \times 100 = \text{Tranfection Efficiency}$$

VII. REFERENCES

1. Sanes J R, J L R Rubenstein and J Nicolas. Use of Recombinant retrovirus to study post-implantation cell lineage in mouse embryos. EMBO. 1986. 5:3133-3142.

VIII. TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Weak β-Gal staining is observed in the cells.	The transfection efficiency is poor.	Check transfection protocol, repeat transfection, or try different transfection methods.
	The incubation time is too short.	Increase the incubation time with the staining solution (up to 24 h).
	The cells are not fixed properly or were improperly washed.	Prepare fresh Fixative Solution and fix cells for 15 minutes. Thoroughly wash cells. Glutaraldehyde will interfere with enymatic activity and color development..
β-Gal staining seems too intense.	The incubation time is too long.	Decrease the incubation time with the staining solution.
Percentage of β-Gal positive cells seems abnormally high.	The cells are confluent.	Confluent cells can lead to false positives because of overlap. Cell confuecy should be less than 80 for staining.
	The incubation time is too long.	Decrease incubation time with the staining solution.
	Cells with high concentration of endogenous β-galactosidase activity used.	Some cells have high endogenous β-Gal activity. Stain nontransfected cells for β-Gal activity as a negative control to determine background.
Blue crystals are observed.	X-Gal has precipitated out of solution (the Staining Solution may be old).	Wash the dish several times with 1X PBS until the crystal number is reduced.
Cells dislodge during staining.	Cells are not fixed properly.	Incubate the cells with fresh Fixative Solution for 15 minutes.
	Loosely adherent type used.	Reduce the number of washes with PBS.