USER GUIDE

invitrogen™
by life technologies™

β-Gal Staining Kit

Catalog number K1465-01

Revision date 9 July 2012
Publication Part number 28-0103

MAN0000624

For Research Use Only.
Not for human or animal therapeutic or diagnostic use.

life technologies™

Contents

Kit Contents and Storage

Kit Components

The kit components, composition, amount and storage conditions are listed in the following table. Enough reagents are provided to stain fifty 60 mm plates.

Component	Composition	Amount	Storage
10X PBS (Phosphate Buffered Saline)	0.017 M KH_2PO_4 0.05 M Na_2HPO_4 1.5 M NaCl, pH 7.4	60 mL	Room temperature, 15°C to 30°C
X-gal (5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside)	provided as a powder	150 mg	–30°C to –10°C
Staining Solution A	400 mM potassium ferricyanide	1.5 mL	–30°C to –10°C
Staining Solution B	400 mM potassium ferrocyanide	1.5 mL	–30°C to –10°C
Staining Solution C	200 mM magnesium chloride	1.5 mL	–30°C to –10°C
10X Fixative Solution	20% formaldehyde 2% glutaraldehyde in 10X PBS	15 mL	–30°C to –10°C
pcDNA3.1/His/LacZ Control Vector	20 µL of vector at 0.5 µg/µL in TE, pH 8.0	10 µg	–30°C to –10°C

Storage and Shipping

The β-Gal Staining Kit is shipped at –20° C. See the preceding table for storage conditions.

Materials Supplied by the User

The following materials are required for use with this kit.

- 37°C incubator
- Phase contrast or light microscope
- N-N-dimethylformamide (DMF)
- 70% glycerol (optional)
- Polypropylene tubes (15 or 50 mL)

Introduction

About the kit

Introduction

The β-Gal Staining Kit from Life Technologies provides reagents required to determine the percentage of cells transfected with a plasmid expressing *lac*Z. *Lac*Z is a bacterial gene often used as a reporter construct in eukaryotic transfection experiments because the gene product, β-galactosidase, is resistant to proteolysis in cellular lysates and its activity is easily assayed. β-galactosidase catalyzes the hydrolysis of β-galactosides, i.e. X-gal, producing a blue color that can be visualized under a microscope.

Experimental Outline

The following table provides a general overview of the steps involved in the β-Gal Staining Kit.

Step	Action
1	Transfect cells with a plasmid expressing *lac*Z.
2	Fix cells to the plates using the fixative solution.
3	Wash cells and incubate with an X-gal containing solution.
4	Examine cells, counting both the number of blue cells and the total number of cells per field of view.
5	Determine the percentage of cells staining blue to estimate transfection efficiency.

Note

This system assumes that all transfected DNA was prepared by the same method. Different methods of preparation will affect the transfection efficiency.

The 10X Fixative Solution contains glutaraldehyde and formaldehyde. Glutaraldehyde is corrosive and a carcinogen and can be absorbed through the skin. Formaldehyde is poisonous and is also absorbed through the skin. Potassium ferricyanide and potassium ferrocyanide are harmful by inhalation, skin contact, and swallowing. **Wear gloves, goggles, lab coats and other protective gear when handling these solutions.**

IMPORTANT!

Dispose of all hazardous substances in accordance with federal, state, and local regulations.

Positive Control

pcDNA3.1His/LacZ (8.6 kb) is provided as a positive control vector for mammalian transfection and expression. It may be used to optimize transfection conditions for your cell line. The *E. coli* gene encoding β-galactosidase is expressed in mammalian cells using the immediate-early promoter from cytomegalovirus.

Methods

How to Use This Kit

Introduction

The following protocol is written for 60 mm plates. The general procedure calls for half the volume of the tissue culture media (e.g. 1 mL for 35 mm wells or plates, 2.5 mL for 60 mm plates, and 5 mL for 100 mm plates).

Before Starting

Be sure to have cells transfected with a *lac*Z construct. Prepare the following solutions:

- Dilute the 10X PBS and 10X Fixative Solutions with distilled water to make 1X solutions. You need 10.5 mL 1X PBS and 3 mL 1X Fixative Solution per 60 mm plate.
- Dissolve 20 mg X-gal in 1 mL DMF to prepare a stock solution. Excess X-gal solution can be stored at −20°C in a light-resistant container for one month. Always use **polypropylene** plastic or glass to make X-gal solutions. Do not use polystyrene.

Procedure

The following procedure is for staining **one 60 mm plate**:

1. Remove the growth medium from the transfected cells and rinse the plate once with 2.5 mL 1X PBS.
2. Fix the cells with 3 mL 1X Fixative Solution for 10 minutes at room temperature.
3. While the plate is in the Fixative Solution, prepare the Staining Solution. Be sure to use **polypropylene** plastic. To prepare the Staining Solution, combine:

25 µL	Solution A
25 µL	Solution B
25 µL	Solution C
125 µL	20 mg/mL X-gal in DMF
2.3 mL	1X PBS

4. Rinse the plate **twice** with 2.5 mL 1X PBS.
5. Add 2.5 mL Staining Solution to the plate. Incubate at 37°C for 0.5–2.0 hours, or longer until the cells stain blue. Rock the plates occasionally to ensure even coverage of the plate.

   Note: If cell confluency is high, do not stain cells for longer than 2 hours before counting. This may result in a greater number of cells staining due to diffusion through gap-junctions. Cells may need to be stained overnight if the confluency or expression level is low. If your 37°C incubator is not humidified, seal the plate with parafilm to prevent it from drying out.
6. Check the cells under a microscope (200× total magnification) for the development of blue color. Count total cells and blue cells in 5–10 random fields of view and use the average to estimate transfection efficiency (see next page for **Calculation**). For long-term storage of stained plates, dispose of the Staining Solution and overlay the cells with 70% glycerol. Store at 4°C.

continued on next page

Calculation

Calculate the percent of cells expressing β-galactosidase (% transfection) with the following formula:

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

IMPORTANT!

This procedure may be adjusted for use with larger or smaller culture plates and/or multiple plates.

Troubleshooting

The following table describes solutions to some possible problems.

Observation	Cause	Solution
No cells stain blue	No X-gal added to staining to staining solution Old X-gal solution used	Add fresh X-gal to plates.
	Cells were not fixed properly	Fix cells for a full 10 minutes.
	Transfection was unsuccessful	Check transfection protocol and repeat transfection using the control plasmid.
		Plasmid preparation was contaminated causing a decrease in transfection efficiency (see **Plasmid Preparation**).
		Try a different method of transfection.
	Construct does not properly express ß-galactosidase	If not using the control vector provided, sequence the construct to ensure that the *lac*Z gene is in-frame with an ATG.
	Cells not washed properly after fixative treatment	Wash cells thoroughly. Glutaraldehyde will interfere with color development.
All cells stain blue	Too much DNA transfected per plate	Transfect cells using less DNA.
	Cells may express endogenous ß-galactosidase like activity	Test by staining non-transfected cells. If endogenous activity is found, use a different cell line.

Plasmid Preparation

Plasmid DNA must be of high quality and free of contaminants. We recommend CsCl gradient ultracentrifugation to purify your construct for transfection. Do not use boiled or alkaline lysis miniprep DNA. Refer to *Current Protocols in Molecular Biology* for large-scale plasmid purification protocols.

Appendix

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com/support**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Notes

Notes

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

life technologies™