In Situ β-Galactosidase Staining Kit

INSTRUCTION MANUAL

Catalog #200384
Revision A

For In Vitro Use Only
200384-12

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In Situ β-Galactosidase Staining Kit

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In Situ β-Galactosidase Staining Kit

MATERIALS PROVIDED

Materials provided[a]	Quantity	Storage conditions[b]
Phosphate-buffered saline (PBS) (10×)	50 ml	4°C
Fixing solution (10×)	2 × 12.5 ml	4°C
Staining buffer	125 ml	4°C
X-Gal solution (100×)	1.25 ml	–20°C[c]
pCMV β-gal control plasmid	15 µl (1µg/µl)	4°C

[a] Contains sufficient reagents for60 standard assays in 60-mm tissue culture dishes.

[b] The solutions are stable for 6 months when stored at the indicated temperatures.

[c] The X-Gal solution is shipped at 4°C. On receipt, store at –20°C.

STORAGE CONDITIONS

X-Gal Solution: –20°C
All Other Components: 4°C

Caution *Wear gloves, goggles, and a lab coat when handling the fixing solution and the staining buffer. To avoid inhaling vapors, prepare the solutions and stain the cells in a fume hood.*

INTRODUCTION

β-Galactosidase is an enzyme that catalyzes the hydrolysis of β-galactosides, including lactose. The β-galactosidase gene functions well as a reporter gene in transfection experiments for two major reasons: its protein product is extremely stable and resistant to proteolytic degradation, and most importantly, the enzyme activity is assayed easily. The In Situ β-Galactosidase Staining Kit provides an easy and rapid method to determine transfection efficiency by indicating β-galactosidase activity in individual intact cells. The transfected cells can be stained in tissue culture dishes without having to remove or manipulate the cells.[1]

The cells are incubated with a glutaraldehyde–formaldehyde fixing solution and then with a staining solution that contains X-Gal. In transfected cells, β-galactosidase cleaves 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-Gal) to produce a blue stain. 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.

Revision A

PREPARING THE FIXING AND STAINING SOLUTIONS

Caution ***Wear gloves, goggles, and a lab coat when handling the fixing solution and the staining buffer. Prepare the solutions in a fume hood.*** *The glutaraldehyde and formaldehyde in the fixing solution and the potassium ferricyanide and potassium ferrocyanide in the staining buffer are harmful if inhaled, swallowed, or absorbed through the skin.*

Note *Use only freshly prepared* ***1×*** *fixing solution and* ***1×*** *staining solution. Prepare just enough of each solution to complete the fixation and staining. Aldehyde-based fixatives oxidize with time, and X-Gal is unstable in the staining buffer and precipitates during long-term storage. See* Table I *for the volumes of fixing solution and staining solution recommended for each type of tissue culture plasticware.*

1. Prepare **1×** PBS by diluting one part of **10×** PBS with 9 parts of dH_2O.

2. Prepare **1×** fixing solution by diluting one part of **10×** fixing solution with 9 parts of **1×** PBS.

3. Prepare **1×** staining solution by diluting one part of **100×** X-Gal solution with 99 parts of staining buffer.

TABLE I

Volumes of Fixing Solution and Staining Solution Recommended for Tissue Culture Plasticware

Plasticware	Well diameter	1× Fixing solution volume	1× Staining solution volume
60-mm dish	60 mm	4.0 ml/dish	2.0 ml/dish
100-mm dish	100 mm	8.0 ml/dish	4.0 ml/dish
150-mm dish	150 mm	18.0 ml/dish	9.0 ml/dish
96-well plate	6.4 mm	0.1 ml/well	0.05 ml/well
48-well plate	10 mm	0.25 ml/well	0.125 ml/well
24-well plate	15 mm	0.5 ml/well	0.25 ml/well
12-well plate	22 mm	1.0 ml/well	0.5 ml/well
6-well plate	35 mm	2.0 ml/well	1.0 ml/well

STAINING TRANSFECTED CELLS IN A TISSUE CULTURE DISH

Caution ***To avoid inhaling vapors, stain the cells in a fume hood.***

Notes *Perform the staining protocol within 24–72 hours after transfection.*

The quantities of the solutions and reagents given in this protocol are optimized for a 60-mm tissue culture dish. See Table I *for solution volumes for dishes of other dimensions.*

1. Aspirate the medium from the cells.

2. Add 4 ml of freshly prepared **1×** fixing solution to the tissue culture dish and incubate the dish for 10 minutes at room temperature.

3. Remove the fixing solution from the dish and gently wash the cells twice with 5 ml of **1×** PBS.

4. Add 2 ml of freshly prepared **1×** staining solution to the tissue culture dish.

5. Incubate the cells between 15 minutes and overnight at 37°C in a humidified incubator.

   Note *The optimal time of incubation depends on the cell type and the transfection efficiency. Observe the intensity of the blue stain in the cells under a microscope and adjust the incubation time accordingly.*

6. Remove the staining solution and wash the cells two or three times with 5 ml of **1×** PBS.

7. Add 2 ml of 1× PBS to the tissue culture dish.

   Note *For long-term storage, cover the dish with a glycerol-based mounting medium and store at 4°C.*

8. Analyze the dish under a light microscope to determine the transfection efficiency. Count the stained and unstained cells in randomly selected fields. The transfection efficiency is the percentage of stained cells in the total population.

TROUBLESHOOTING

Observation	Suggestion(s)
Weak or no blue stain is observed in the cells	The transfection efficiency is poor. Repeat the transfection, optimizing the cells for maximum transfection efficiency, or try different transfection methods
	Increase the incubation time with the staining solution (up to 24 hours)
	The cells are not fixed properly. Use freshly prepared **1×** fixing solution and fix cells for a full 10 minutes
Blue stain in the cells is too intense	Decrease the incubation time with the staining solution
Percentage of blue-stained cells is abnormally high	The cells are too confluent, leading to false positives because of cell overlap. Stain tissue cultures in which the cells are less confluent
	The incubation time with the staining solution is too long, leading to excessive blue precipitation. Decrease the incubation time with the staining solution
	Some cells have high endogenous β-galactosidase activity. Stain nontransfected cells for β-galactosidase activity to compare with the transfected cells
Blue crystals are observed in the tissue culture dish	The staining solution is old and the X-Gal has precipitated out of solution. Wash the dish several times with **1×** PBS until the crystal number is reduced
Cells dislodge during staining	Cells of a loosely adherent type are lifting from the dish. Reduce the number of washes with PBS
	The cells are not fixed properly. Incubate the cells with **1×** fixing solution for a full 10 minutes

COMPOSITION OF KIT SOLUTIONS

10× Fixing Solution 20% (v/v) formaldehyde 2% (v/v) glutaraldehyde PBS	**100× X-Gal Solution** 100 mg/ml 5-Bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-Gal) dimethyl sulfoxide (DMSO)
Staining Buffer 5 mM potassium ferricyanide 5 mM potassium ferrocyanide 2 mM magnesium chloride 10% dimethyl sulfoxide (DMSO) buffer stabilizer PBS	**10× PBS** 1370 mM sodium chloride 26 mM potassium chloride 100 mM disodium phosphate 18 mM potassium phosphate pH adjusted to 7.4 with HCl

REFERENCE

1. Sanes, J. R., Rubenstein, J. L. and Nicolas, J. F. (1986) *Embo J* 5(12):3133-42.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

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QUICK-REFERENCE PROTOCOL

- Prepare **1×** PBS by diluting 1 part of **10×** PBS with 9 parts of dH_2O
- Prepare **1×** fixing solution by diluting 1 part of **10×** fixing solution with 9 parts of **1×** PBS
- Prepare **1×** staining solution by diluting 1 part of **100×** X-Gal with 99 parts of staining buffer
- Aspirate the medium from the cells
- Add 4 ml of freshly prepared **1×** fixing solution to the tissue culture dish and incubate for 10 minutes at room temperature
- Remove the fixing solution from the dish and gently wash the cells twice with 5 ml of **1×** PBS
- Add 2 ml of freshly prepared **1×** staining solution to the tissue culture dish
- Incubate the cells between15 minutes and overnight at 37°C in a humidified incubator
- Remove the staining solution and wash the cells two or three times with 5 ml of **1×** PBS
- Add 2 ml of **1×** PBS to the dish
- Analyze the specimens under a light microscope and determine the transfection efficiency