Product Manual

β-Galactosidase Staining Kit

Catalog Number

AKR-100 **75 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

The delivery of reporter genes into cells has become the primary means by which researchers study gene function and gene expression regulation. LacZ is a commonly used reporter gene in transfection experiments because the gene product, β-galactosidase, is very stable and resistant to proteolytic degradation and easily assayed. The β-galactosidase Staining Kit provides an easy-to-use and efficient method to determine the transfection efficiency and expression of LacZ gene. β-galactosidase catalyzes the hydrolysis of X-gal, which produces a blue color in cells expressing the transfected gene. Each kit provides sufficient quantities to perform up to 75 assays in 35 mm dishes.

Related Products

1. CBA-230: Cellular Senescence Assay Kit (SA β-gal Staining)
2. CBA-231: 96-Well Cellular Senescence Assay (SA β-gal Activity)

Kit Components

1. 100X Fixing Solution (Part No. 40010): One tube – 1.5 mL of 25% Glutaraldehyde
2. Staining Solution A (Part No. 40011): One tube – 1.5 mL of 500 mM Potassium Ferrocyanide
3. Staining Solution B (Part No. 40012): One tube – 1.5 mL of 500 mM Potassium Ferricyanide
4. Staining Solution C (Part No. 90013): One tube – 1.5 mL of 200 mM $MgCl_2$
5. X-gal Solution (Part No. 40014): Three tubes – 1.5 mL of 40mg/mL X-gal in DMF for each tube

Materials Not Supplied

1. PBS
2. 37ºC Incubator
3. Light microscope
4. Cells or tissue samples expressing LacZ

Storage

Store X-gal solution protected from light at -20ºC, and other kit components at 4ºC.

Preparation of Reagents

- 1X Fixing Solution: Prepare a 1X Fixing Solution by diluting the provided 100X stock 1:100 in 1X PBS. Store the diluted solution at room temperature for up to six months.
- Cell Staining Working Solution: Prepare FRESH cell staining working solution based on the number of samples. The chart below is suggested for samples in 35 mm plate, and may be modified accordingly to suit other culture plate sizes.

Reagents	1 plate (35 mm)	5 plate (35 mm)	10 plate (35 mm)
Staining Solution A	20 µL	100 µL	200 µL
Staining Solution B	20 µL	100 µL	200 µL
Staining Solution C	20 µL	100 µL	200 µL
X-Gal Solution	50 µL	250 µL	500 µL
1X PBS	1.89 mL	9.45 mL	18.9 mL
Total	**2 mL**	**10 mL**	**20 mL**

Staining Protocol for a 35 mm Plate

1. Aspirate the medium from the LacZ gene transfected or infected cells.
2. Wash the cells twice with 3 mL of 1X PBS and aspirate the final wash.
3. Add 2 mL of 1X Fixing Solution. Incubate at room temperature for 5 minutes.
4. Remove the fixing solution and wash the fixed cells three times with 3 mL of 1X PBS.
5. Aspirate the final wash, and completely cover cells by adding 2 mL of freshly prepared Cell Staining Working Solution.
6. Incubate the cells at 37ºC protected from light for 1 hr to overnight.
7. Remove the Cell Staining Working Solution, then wash the stained cells twice with 3 mL of 1X PBS and store cells in 1X PBS. For long-term storage, overlay the cells with 1X PBS containing 20% Glycerol. Store at 4ºC.

Note: Excess amount of salt crystals can be removed by briefly incubating the stained sample with DMSO.

8. Count the blue stained cells using light microscope. To determine transfection or infection efficiency, calculate the ratio of blue stained cells to total cells.

Example of Results

The following figures demonstrate typical results with the β-Galactosidase Staining Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

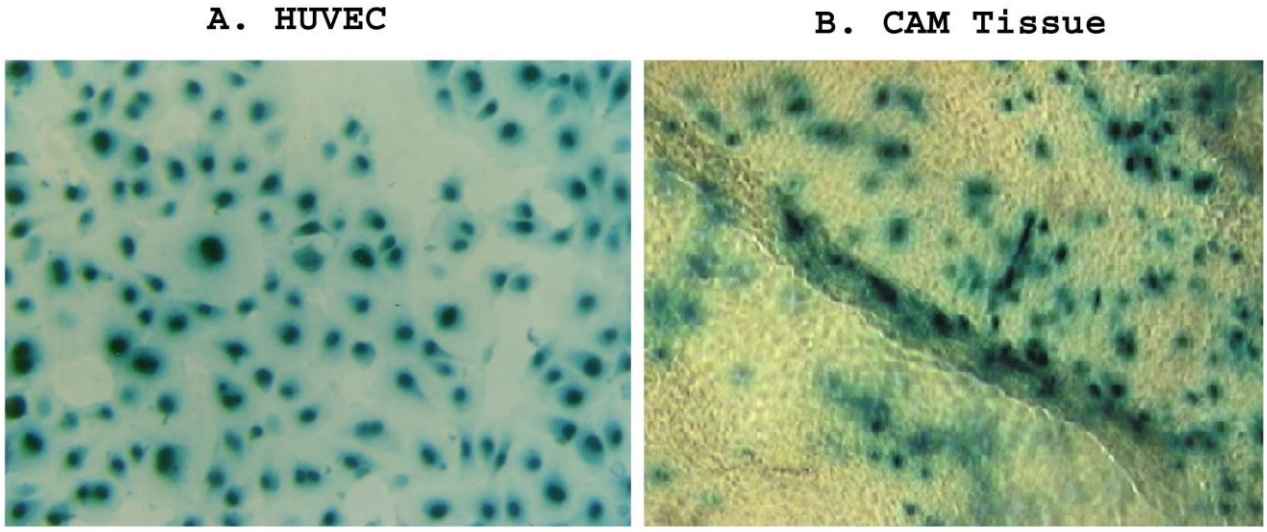

Figure 1: X-gal staining of infected HUVEC cells and chick CAM tissue. A. HUVEC cells were infected with purified Ad-β Gal at 50 MOI (multiplicity of infection). X-gal staining was performed after 48 hr infection period. B. Purified Ad- β Gal was injected intravenously into a 10-day old chick embryo, after three days, X-gal staining was performed on the CAM (chick chorioallanoic membrane) tissue.

References

1. Current Protocols in Molecular Biology, John Wiley & Sons Press.

Recent Product Citations

1. Kadoya, H. et al. (2020). Klotho is a novel therapeutic target in peritoneal fibrosis via Wnt signaling inhibition. *Nephrol Dial Transplant*. pii: gfz298. doi: 10.1093/ndt/gfz298.
2. Rani, S., et al. (2017). Senescence in the lesional fibroblasts of non-segmental vitiligo patients. *Arch Dermatol Res*. **309**(2):123-132. doi:10.1007/s00403-016-1713-0
3. Ge, X. et al. (2015). Mitochondrial catalase suppresses naturally occurring lung cancer in old mice. *Pathobiol Aging Age Relat Dis.* **5**:28776.
4. Xu, X. et al. (2015). Aberrant activation of TGF-β in subchondral bone at the onset of rheumatoid arthritis joint destruction. *J Bone Miner Res.* doi: 10.1002/jbmr.2550.
5. Black, S.A. et al. (2008). TGFß1 stimulates connective tissue growth factor (CCN2/CTGF) expression in human gingival fibroblasts through a RhoA-independent, Rac1/Cdc42-dependent mechanism: statins with forskolin block TGFß1-induced CCN2/CTGF expression. *J. Biol. Chem.* **283**:10835-10847.

Warranty

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