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Product Information

β-Galactosidase Reporter Gene Staining Kit

Product Number **GALS**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Reporter genes are “markers” widely used for analysis of mutationally altered genes as well as gene regulation. The expressed reporter genes are detected by biochemical activity assays, by immunological analysis, or by histochemical staining of tissue sections or cells.

The β-galactosidase gene, *LacZ*, from *E. coli*, is often used as a reporter gene in eukaryotic transfection. β-Galactosidase catalyzes the hydrolysis of various β-galactosides. Substrates designed to produce chromogenic, fluorescent, or chemiluminescent products are used for β-galactosidase activity detection. The substrate used for histological staining, X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), generates an indigo-blue color in cells which express the *E. coli* β-galactosidase. The successful enzyme expression is visualized under a microscope. This test provides a method for determining the percentage of cells transfected with the plasmid expressing *LacZ* or for visualizing the specific expression of the reporter gene in tissue sections.

X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galacto-pyranoside) is applied to fixed tissue sections or cells in PBS solution containing magnesium chloride ($MgCl_2$), potassium ferricyanide ($K_3Fe(CN)_6$) and potassium ferrocyanide ($K_4Fe(CN)_6$). The substrate penetrates the cells and is hydrolyzed by the β-galactosidase. The indolyl moiety obtained is oxidized to an indoxyl in a reaction catalyzed by the mixture of ferricyanide/ ferrocyanide. The indoxyl moiety undergoes dimerization and forms an indigo blue derivative.

Reagents

The kit is sufficient for 100 tests in 3.5 cm tissue culture plates.

10× PBS, Product No. P6724 70.2 mM Na_2HPO_4, 14.7 mM KH_2PO_4, 1.37 M NaCl, and 26.8 mM KCl	60 ml
10× Fixation Buffer, Product No. F1797 20% formaldehyde and 2% glutaraldehyde in 10× PBS	15 ml
Reagent A, Product No. R5397 200 mM $MgCl_2$	1.5 ml
Reagent B, Product No. R5272 400 mM potassium ferricyanide	1.5 ml
Reagent C, Product No. R5147 400 mM potassium ferrocyanide	1.5 ml
X-Gal Solution, Product No. X3753 40 mg/ml	4 ml

Reagents and Equipment Required but not Provided

- 37 °C incubator
- Phase contrast or light microscope
- 15 ml or 50 ml polypropylene tubes, Product No. C3048 (15 ml) or C8171 (50 ml)
- 70% glycerol solution, prepared with Product No. G5516 (optional)
- Calcium Phosphate Transfection Kit, Product No. CAPHOS (optional)
- ESCORT™ Transfection Reagent, Product No. E9770 (optional)

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Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices

Preparation Instructions
Dilute 10× PBS 10-fold with 0.2 µm filtered water to prepare 4 ml/plate of 1× PBS.

Dilute 10× Fixation Buffer 10-fold with 0.2 µm filtered water to prepare 1 ml/plate of 1× Fixation Solution.

Storage/Stability
The kit ships on dry ice and storage at –20 °C is recommended. After thawing, store 10× PBS solution at 2–8 °C.

Procedure
All steps must be performed in a laminar flow hood.

A. Transfect the cells with β-galactosidase encoding plasmid using Calcium Phosphate Transfection Kit (Product No. CAPHOS) or ESCORT Transfection Reagent (Product No. E9770). For control, transfect cells without DNA.
Note: The efficiency of the transfection is largely dependent on the construct and quality of the DNA preparation. The duration of the transfection must be optimized by the researcher.

B. Analyze the β-galactosidase expression 40–72 hours post-transfection.
Note: The volumes indicated are suitable for transfections in 3.5 cm plates. Adjust the volumes for different size plates.

1. Aspirate the growth medium from the transfected cells.
2. Wash cells twice with 1 ml of 1× PBS. Remove the wash solution entirely with aspiration.
3. Add 1 ml of 1× Fixation Solution and incubate 10 minutes at room temperature.
4. During the fixation process prepare the Staining Solution in a polypropylene tube (see Table 1). Prepare sufficient Staining Solution for N+1 plates where N is the number of plates to be evaluated.

Table 1.
Staining Solution

Component	Amount per 1 plate
Reagent A	10 µl
Reagent B	10 µl
Reagent C	10 µl
X-Gal solution (40 mg/ml)	25 µl
1× PBS	945 µl
Total volume	1 ml

5. Rinse the cells twice with 1 ml of 1× PBS.
6. Add 1 ml of Staining Solution to the plate. Ensure even coverage of the plate.
7. Incubate at 37 °C for 0.5–2 hours or longer, until the cells stain blue. In the event a longer staining period is needed, seal the plate with Parafilm® to prevent it from drying out. The exact incubation time has to be optimized.
8. Observe the cells under the microscope. Count the cells and calculate the percent of cells expressing β-galactosidase.
9. For long-term storage of stained plate, remove the Staining Solution, overlay cells with 70% glycerol and store at 4 °C.

References
Sambrook, J., and Russel, D., in Molecular Cloning: A Laboratory Manual, Third edition, Cold Spring Harbor Laboratory Press, (Plainview, New York: 2001), pp. 17.97-17.99.

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