BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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LacZ Staining Kit (Cat #: E-105)

COMPONENTS: X-gal Buffer- 150 ml, store at 4ºC (300-500 assays)
Ferricyanide- 10 ml, store at 4ºC
Ferrocyanide- 10 ml, store at 4ºC
X-gal- 4 ml, store at -20ºC

PRODUCT DESCRIPTION: The bacterial enzyme β-galactosidase (LacZ) is a widely used reporter enzyme, the activity of which can be conveniently measured spectrophotometrically (J. Med. Chem. 7:574,1964). The LacZ Staining Kit is based on the histochemical substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal) for in situ staining of transgenic tissues or monolayer cells transiently or stably expressing GAL. The GAL staining kit requires minimal steps for localization of LacZ, allowing easy assessment of gene expression in animal tissue and transfected cells. Kit components are stable for at least one year if stored and handled properly.

PROTOCOL:

Fixation solution (not included in the kit):

For best result, prepare a 4% paraformaldehyde solution prior to assays. In the fume hood, add 4 g of paraformaldehyde powder to 100 ml of normal phosphate-buffered saline (PBS). Stir solution with heat on (60-80°C) to dissolve powder. This preparation may take up to one hour for complete dissolution of the paraformaldehyde powder. Cool down the fixation solution to room temperature prior to use.

Xgal working solution:

Prepare a fresh Xgal working solution for staining. Thaw Xgal solution and vortex solution thoroughly to dissolve precipitates. Caution should be taken to minimize light exposure. Per ml of Xgal Buffer, add in the following order: 60 µl Ferricyanide, 60 µl Ferrocyanide, and 25 µl Xgal. Mix contents by agitation. Use the working solution immediately.

Fixation and Staining:

1. For monolayer cells and frozen tissue sections: Aspirate off liquid completely. Rinse cells and tissue sections once with phosphate-buffered saline (PBS) and remove PBS. Immerse samples in freshly prepared paraformaldehyde solution for 5 min. Aspirate off paraformaldehyde completely and wash samples three times (5 min each wash) with PBS. Aspirate off PBS completely after each wash.

2. Immerse samples in enough freshly prepared X-gal working solution. Cover the samples and incubate in a humidified 37°C incubator until blue color become visible under microscope (several hours may be required for maximal staining).

3. Terminate staining by replacing X-gal working solution with PBS. The samples are now ready for imaging.

Additional information:

Paraformaldehyde is toxic and should be handled in the fume hood with caution. X-gal is prepared in formamide, a skin- and mucous membrane-irritating solvent. Please visit the product webpage or contact us for SDS information.