BIOMEDICAL RESEARCH SERVICE CENTER
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β-Galactosidase (GAL) Staining Kit (Cat #: E-105)

COMPONENTS:	10x GAL Staining Buffer:	15 ml, store at 4ºC (300-500 assays)
	Ferricyanide:	10 ml, store at 4ºC
	Ferrocyanide:	10 ml, store at 4ºC
	X-gal:	4 x 1 ml, store at -20ºC (shielded from light)

PRODUCT DESCRIPTION: The bacterial enzyme β-galactosidase (GAL) is a widely used reporter enzyme, the activity of which can be conveniently measured spectrophotometrically (J. Med. Chem. 7:574,1964). The GAL 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 GAL, allowing easy assessment of GAL expression in animal tissue and transfected cells. Reagents are stable for ~one year if handled and stored properly.

PROTOCOL:

Fixation solution (not included in the kit): For best result, prepare a 4% paraformaldehyde solution prior to assay.

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.

Staining solution: Prepare fresh enough staining solution prior to assay. Note that thawed Xgal should be vortexed thoroughly to dissolve precipitates and should be shielded from light.

For preparation of 1 ml staining solution: mix 0.9 ml dH_2O, 0.1 ml 10x GAL Staining Buffer, 60 µl Ferricyanide, 60 µl Ferrocyanide, and finally 25 µl Xgal. Mix contents thoroughly by vortexing. Use the freshly prepared staining solution immediately.

1. For monolayer cells and frozen tissue section: Aspirate off cell culture medium. Rinse cells and tissue section once with PBS and remove PBS. Immerse cells or tissue section in freshly prepared paraformaldehyde solution for 5 min.

2. Aspirate off paraformaldehyde completely, and wash samples three times (5 min each wash with gentle agitation) with PBS. Aspirate off PBS completely after each wash.

3. Immerse fixed cells or tissue section in enough staining solution. Keep sample in a humidified 37°C incubator until blue cells become visible under microscope (several hours may be required).

4. Terminate staining by replacing staining solution with PBS. The sample is now ready for imaging.

Additional information:

- 4% paraformaldehyde is required for cell/tissue fixation, but is not included in the kit. Paraformaldehyde is extremely toxic, and should be handled in the fume hood with caution.
- X-gal solution contains formamide, a skin- and mucous membrane-irritating organic solvent. Please visit the product webpage or contact us for MSDS information.
- For preparation of 20x PBS, please visit http://www.bmrservice.com/TechNotes.html. Dilute 20x PBS 20-fold with dH_2O to obtain 1x PBS for cell washing.