COS-0104

β-Galactosidase Staining Kit

(To stain 20 X 12-well plates or the equivalent volume of 6 or 24-well plates, Store at -20°C)

Introduction:

The E. coli LacZ gene is a commonly used reporter gene for testing the expression efficiency of vector mediated gene transfer. It is also used frequently to study gene promoter regulation. LacZ encodes β-galactosidase, an almost perfectly ideal choice for expression due to being very stable, resistant to proteolytic degradation, able to utilize multiple substrates and easily assayed in situ. AkrivisBio's β-Galactosidase Staining Kit is a simple, sensitive assay which utilizes X-Gal a colorless substrate, resulting in an intense blue insoluble indigo product easily visualized under microscopy. It contains sufficient reagents to stain 20 X 12-well plates or the equivalent volume of 6 or 24-well plates.

Kit Components:

Fixative	125 ml	NM	COS-0104A
X-Gal	lyoph	Green	COS-0104B
Staining Buffer	125 ml	WM	COS-0104C
Staining Supplement	1.5 ml	Red	COS-0104D

Storage and Handling:

Store the unopened kit at -20°C. Once opened all components can be stored at 4°C except the X-Gal, which should be stored at -20°C.

User Supplied Materials:

PBS ~ 1 L
DMSO or DMF
Polypropylene tubes such as Eppendorf and similar capped conical micro tubes. **DO NOT USE Polystyrene containers.**
70% glycerol

Staining Protocol:

A. General Consideration:

This protocol is designed for wells in a 12-well culture plate. If using other plates, increase or decrease volumes proportionately.

B. Reagent Preparation:

Dissolve 20 mg X-Gal in 1 ml DMSO or DMF providing a 20X stock solution. Unused X-Gal solution can be stored at –20°C for up to one month.
Note: Always use polypropylene container or glass to make and store X-gal. Do not use polystyrene. If precipitation occurs, simply warm up the solution to redissolve the precipitate.

C. Staining Protocol:

1. Remove culture medium and wash cells once with 1 ml of PBS.
2. Fix the cells with 0.5 ml of Fixative for 10 - 15 min at room temperature.
3. While the cells are in the Fixative, prepare the following Staining Solution, using polypropylene tubes only. Each well will require 0.5 ml. Prepare sufficient Staining Solution for the number of wells to be stained using:

Staining Buffer:	470 µl
Staining Supplement:	5 µl
20 mg/ml X-gal in DMF	25 µl

4. Wash the cells two times with 1 ml of PBS.
5. Add 0.5 ml of the Staining Solution to each well, cover the plate and incubate overnight at 37°C.
6. Next day, observe cells under a microscope at ~ 200X magnification for development of blue color.
7. For long-term preservation of stained plates, remove the Staining Solution and overlay the cells with 70% glycerol. Store at 4°C.

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

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