BetaBlue™ Staining Kit

About the Kit

BetaBlue™ Staining Kit	100ml	71074-3

Description

Gene transfer is often monitored using -galactosidase as the reporter. The BetaBlue™ Staining Kit provides direct visualization of ß -galactosidase expression in isolated cells, tissues or intact organisms. The kit contains solutions of the substrate X-gal (5-bromo4-chloro-3-indolys- ß -D-galactopyranoside) and Reaction Buffer optimized for rapid, sensitive histochemical staining with minimal background. Exceptional staining with BetaBlue Staining Kit enables quick, accurate determination of transfection efficiencies, assessment of stable cell line generation, and transgene expression in tissue slices or whole mounts of transgenic animals.

Components

2 × 50 ml	BetaBlue Reaction Buffer
3 × 1 ml	X-gal Solution, 40mg/ml in DMSO

Storage

The BetaBlue Assay Kit should be stored at 4°C. For storage longer than 3 months, place the X-gal at – 20 °C.

Additional reagents/supplies needed

Phosphate buffered saline (PBS)
Fixation solution (e.g. 4% paraformaldehyde in phosphate buffered saline)

BetaBlue Staining

The following procedure describes a sensitive general detection assay for ß -galactosidase expression in mammalian cells. Modification of the procedure may be required for extremely high or low expression levels, alternate plate formats or tissues that are difficult to penetrate.

Staining of transiently transfected cells in 35 mm dishes

1. Transfect cells using a suitable transfection reagent (e.g. GeneJuice™ Transfection Reagent Cat. No. 70967-3) with a high purity DNA (e.g. purified with Mobius™ or UltraMobius™ Plasmid Kits) construct capable of expressing -galactosidase. Begin BetaBlue staining between 24 and 72 h after transfection.
2. Include a negative control (e.g. mock transfected cells or non-transgenic tissue).
3. Prepare BetaBlue staining solution by calculating the appropriate volume needed (see page 2) for the number of wells used. Thaw the X-gal solution at room temperature and dilute 1:40 in BetaBlue Reaction Buffer. Mix thoroughly by inversion.

Note: *Tissues that are difficult to penetrate (e.g. tissue slices, whole mounts) may require inclusion of 0.02% NP-40 in the staining solution to aid in tissue penetration.*

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Suggested volume of BetaBlue staining solution

Culture format	BetaBlue staining solution volume
8-well chamber slide	100 µl/well
4-well chamber slide	250 µl/well
2-well chamber slide	500 µl/well
96-well plate	100 µl/well
24-well plate	250 µl/well
12-well plate	500 µl/well
6-well plate	2.5 ml/well
35 mm dish	2.5 ml
60 mm dish	5 ml
100 mm dish	10 ml
T-75 flask	10 ml

4. Aspirate culture medium from cells. Wash the cells twice with PBS.
5. To fix cells, add 2.5 ml of 4% paraformaldehyde (in PBS) incubate for 15 min at room temperature. Alternative fixatives may be used as needed.
6. Remove the fixative and wash cells 4 times with PBS.
7. Gently add 2.5 ml of prepared BetaBlue staining solution (with X-gal) to the 35 mm dish and incubate at 37 °C. Avoid using a tissue culture incubator as the $C0_2$ may alter the pH, resulting in higher background.

Note: *For cells expressing high levels of ß-galactosidase, a cytoplasmic blue color will be apparent within 15 minutes. For most cell types, BetaBlue staining will be visible within 3 hours at 37 °C. Longer incubation times may be required for hard to transfect or weakly expressing cell lines.*

8. To stop color progression, remove the BetaBlue staining solution and wash cells 4 times with PBS. View stained cells with a microscope or take a photograph. For long term storage, remove the PBS and replace with 3 ml of 15% glycerol (v/v) in PBS and store at 4 °C.

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