Detection of the beta-galactosidase Reporter Gene in Transfected Eukaryotic Cells

Protocol

This protocol is for the Detection of the beta-galactosidease Reporter Gene in Transfected Eukaryotic Cells

10X PBS buffer (pH 7.4): 1.37 M NaCl, 0.27 M KCl, 1 M Na_2HPO_4, 0.02 M K_2HPO_4.

Fixation buffer (pH 7.4): 1X PBS buffer and 0.25% glutardialdehyde.

Staining buffer, prepare immediately before use as follows:

Stock solutions	Volume per 10 ml staining buffer	Final concentration
1 M MgCl2	20 µl	2 mM
0.5 M K4Fe(CN)63H2O	100 µl	5 mM
0.5 M K3Fe(CN)6	100 µl	5 mM
X-Gal Solution, ready-to-use	500 µl	1 mg/ml
10X PBS buffer (pH 7.4)	9.28 ml	diluted 10-fold

Staining procedure:

1. Wash the cells twice with cold 1X PBS buffer. Adhered cells can be washed in the transfection plates, suspension cells should be pelleted before washing.
2. Fix the cells with Fixation buffer for 10 minutes at room temperature while gently rocking the plate. Use 150 µl of the Fixation buffer for each well of a 24-well plate.
3. Wash the cells twice with cold 1X PBS buffer.
4. Stain the cells with freshly prepared Staining buffer for 2-20 hours at 37°C. Use 200 µl of Staining buffer for each well of a 24-well plate.
5. Count dark blue cells.

North America
Technical Services:
techservice.genomics@thermofisher.com
Customer Services:
customerservice.genomics@thermofisher.com
Tel 800 235 9880
Fax 800 292 6088

Europe and Asia
Technical Services:
techservice.emea.genomics@thermofisher.com
Customer Services:
customerservice.emea.genomics@thermofisher.com

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific