

Beta-Galactosidase Staining Kit

Product Name	Quantity	Product No.
β-Gal Staining Kit	100 Assays in 35 mm wells	MIR 2600

1.0 DESCRIPTION

1.1 General Information

The delivery of expression vectors or reporter vectors into cells has become the primary means by which researchers study gene function and gene expression regulation. Since different cell types transfect at varying efficiencies, it is important to determine the number of cells expressing the transfected gene. In many types of studies, this can be easily accomplished by transfecting a β-galactosidase expression vector. The efficiency of transfection and expression of the β-galactosidase reporter gene can be monitored using Mirus' β-Gal Staining Kit.

Using the Mirus Bio' β-Gal Staining Kit, the efficiency of β-galactosidase expression can be easily determined *in situ* following transient or stable transfections in cultured cells. This kit can also be used to visualize β-galactosidase expression in tissue sections following *in vivo* transfections. The simple colorimetric assay allows direct visualization of individual cells expressing the reporter gene. The β-Galactosidase Staining Kit provides all the reagents necessary for efficient and reliable determination of β-galactosidase expression. Each kit (MIR 2600) provides sufficient quantities to perform up to 100 assays in 35 mm wells.

NOTE: This kit is generally not recommended for paraffin embedded tissue sections.

1.2 Kit Components

Component	Volume*
Cell Fixative Reagent	8 ml
Cell Staining Solution	200 ml
X-Gal Reagent	4 ml

*Excess reagent is supplied.

1.3 Required Materials (not supplied)

General reagents:

Phosphate-Buffered Saline (PBS): 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.4.

Equipment: Bright-field or phase-contrast microscope

1.4 Specifications

Concentration: Cell Staining Solution: proprietary
 X-Gal Reagent: 2 mg/ml in dimethyl formamide
 Cell Fixative Reagent: 50% Gluteraldehyde

Storage: Store the Cell Fixative Reagent at 4°C. Store the Cell Staining Solution and the X-Gal Reagent at -20°C.

Stability: 6 months when stored properly

2.0 PROCEDURE

2.1 Preparation of Supplied Solutions

A. Cell Fixative Working Solution

NOTE: Prepare fresh immediately prior to each use. Do not reuse solution.

1. Determine the volume of Cell Fixative Working Solution needed (Mirus recommends 1-2 ml per 35 mm well).
2. Dilute 1 part of Cell Fixative Reagent with 24 parts of PBS.
3. Mix thoroughly.

B. Cell Staining Working Solution

NOTE: Prepare fresh immediately prior to each use. Do not reuse solution.

1. Determine the volume of Cell Staining Working Solution needed (Mirus recommends 1-2 ml per 35 mm well).
2. Add 20 μ l of X-Gal Reagent to every 1 ml of Cell Staining Solution.
3. Mix thoroughly.

2.2 β -Galactosidase Staining For Transfected Cells (in 35 mm wells)

NOTE: Tissue culture plastic may interfere with optimal direct microscopic viewing of transfected cells. For better visualization, we recommend plating the cells on poly-D-lysine (PDL) coated coverslips, which can be mounted for microscopic observation

NOTE: Visually check cells between steps to ensure they are still attached to the well. Wash the cells gently throughout the procedure to ensure cell adherence.

1. Transfect cells with a β -galactosidase expression vector according to manufacturer's instructions.
2. After the optimized post-transfection incubation period, aspirate the media from the cells.
3. Wash the cells twice with approximately 2 ml of PBS per well and aspirate the final wash.
4. Add approximately 2 ml of the Cell Fixative Working Solution to each well.
5. Incubate at room temperature for 2-5 minutes.
6. Remove the Cell Fixative Working Solution.
7. Wash the cells 3 times with PBS (2 ml per 35 mm well) and aspirate the final wash.
8. Completely cover the cells by adding 1-2 ml of the Cell Staining Working Solution to each well.
9. Incubate the cells at 37°C protected from light for 20 minutes to 16 hours.

NOTE: Carefully monitor the staining process by periodically checking the cells under a light microscope. Continue staining until the desired staining intensity is reached. Examine the cells at regular time points to avoid overstaining.

10. After incubation, remove the Cell Staining Working Solution, then wash the cells 3 times with water or PBS.
11. Store cells in 2 ml per well of water or PBS.
12. Count the blue stained cells using light microscopy (bright-field or phase-contrast). To determine transfection efficiency, calculate the ratio of blue stained cells to total cells.

2.3 β -Galactosidase Staining for Tissue Sections

NOTE: This kit can be used for direct visualization of β -Galactosidase expression in combination with antibodies coupled to functional bacterial β -galactosidase for immunohistochemical staining applications.

NOTE: Paraffin-embedded sections must be rehydrated through a series of decreasing ethanol concentrations (i.e., 3 minutes each in 100%, 80%, 40%, and 20% ethanol, then water). Frozen sections must be thoroughly dried before use.

1. Prepare tissue sections and (if necessary) perform primary antibody incubations according to standard protocols.¹⁻⁴
2. Wash the tissue sections with PBS at least 3 times for 5 minutes each.
3. Completely cover the specimen with Cell Staining Working Solution.
4. Incubate the specimen in a humidified chamber at 37°C for 20 minutes to 16 hours in the dark. Carefully monitor the staining progress by periodically checking under a light microscope. Continue staining until the desired staining intensity is reached.

5. Wash the specimen 3 times with water or PBS. Use a counterstain if desired. Stained sections can be stored for a short time in water or PBS. For extended storage, stained sections should be protected from drying with a suitable mounting medium.
6. View stained tissue sections using light microscopy.

3.0 TROUBLESHOOTING

3.1 Suboptimal Staining of Cells

- **Understaining of cells**

1. Continue staining until the desired staining intensity is reached by incubating at 37°C protected from light. Optimal incubation time can range from 20 minutes to 16 hours.
2. Ensure the DNA used for transfection is of pure quality and functional.
3. Confirm optimized transfection parameters were used to achieve high transfection efficiency. For specific concerns regarding efficiency, please contact our technical support team at 888.530.0801 or techsupport@mirusbio.com.

- **Overstaining of cells**

1. Staining Solution was left on cells too long. Repeat experiment and carefully monitor the staining progress by periodically checking under a light microscope.
2. Unhealthy cells can take up the Staining Solution and cause false positive cells. If necessary, perform another transfection in parallel using a different cell type to ensure cells stain as expected.
3. Prolonged incubation may cause stain to “bleed” into surrounding cells and cause false positive cells.

- **Cells peel away from the well plate**

1. Cells were too confluent at time of transfection. Plate cells at a lower density and repeat the experiment. The recommended cell density for most cell types at the time of transfection is 50-70% confluence.
2. Cell Fixative Solution was not properly diluted. Perform a 1:25 dilution in PBS. For example, to make 50 ml of Cell Fixative Working Solution, dilute 2 ml of Cell Fixative Solution in 48 ml of PBS.
3. Cells were not fixed properly. Wash the cells with PBS two times to remove the media. Add enough Cell Fixative Working Solution to completely cover the cells and incubate 2-5 minutes at room temperature.
4. Cells were treated too vigorously during wash steps. Treat cells gently throughout procedure.

3.2 High Cellular Toxicity

- **Excessive amount of transfection reagent /DNA complex mixture used in the transfection**

High transfection efficiencies and low cytotoxicity can be achieved when using the appropriate transfection reagent specific for each cell type. Please see Related Products section or contact Technical Support at 888.530.0801 or techsupport@mirusbio.com for assistance.

- **Suboptimal cell density (% confluence) at the time of transfection**

The recommended cell density for most cell types at the time of transfection is 50-70% confluence. However, it may be necessary to determine the optimal cell density for different cell types in order to maximize transfection efficiency. Maintain this density in future experiments for reproducibility.

- **Poor quality of transfecting DNA**

Use double-stranded, cesium chloride-purified DNA if commercial methods have not worked satisfactorily. Remove any traces of endotoxin (Lipopolysaccharide, LPS) using Mirus' MiraCLEAN[®] Endotoxin Removal Kit (Product # MIR 5900).

- **Cell morphology has changed**

If the passage number of the cells is too high or too low, they can be more sensitive to the transfection process. Maintain a similar passage number between experiments to ensure reproducibility.

For specific questions or concerns, please contact Mirus' Technical Support at 888.530.0801 or techsupport@mirusbio.com

4.0 REFERENCES

1. Alton, E.W.F.W., et al. (1993) Nature Gen. 5: 135.
2. Bondi, A., et al. (1982) Histochemistry 76: 153-158.
3. Harlow, E. and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
4. Holzmann, B. and Johnson, J.P. (1983) J. Immunol. Meth. 60: 359-367.

5.0 RELATED PRODUCTS

For endotoxin removal from DNA:*

MiraCLEAN[®] Endotoxin Removal Kit (Product #5900)

For DNA tracking studies:

Label IT[®] Tracker[™] Intracellular Nucleic Acid Localization Kit (Product # MIR 7010,7011,7012,7013,7014,7015)

Transfection reagents:*

TransIT[®]-LT1 Transfection Reagent (Product # MIR 2300)

TransIT[®]-LT2 Transfection Reagent (Product # MIR 2400)

TransIT[®]-Express Transfection Reagent (Product # MIR 2000)

TransIT[®]-HeLaMONSTER[®] Transfection Kit (Product # MIR 2900)

TransIT[®]-293 Transfection Reagent (Product # MIR 2700)

TransIT[®]-Keratinocyte Transfection Reagent (Product # MIR 2800)

TransIT[®]-CHO Transfection Kit (Product # MIR 2170)

TransIT[®]-3T3 Transfection Kit (Product # MIR 2180)

TransIT[®]-COS Transfection Kit (Product # MIR 2190)

TransIT[®]-Insecta Transfection Reagent (Product # MIR 2200)

TransIT-Neural[®] Transfection Reagent (Product # MIR 2140)

TransIT-TKO[®] siRNA Transfection Reagent (Product # MIR 2150)

TransIT[®]-siQUEST[™] siRNA Transfection Reagent (Product # MIR 2110)

TransIT[®]-Oligo Transfection Reagent (Product # MIR 2160)

In Vivo Gene Delivery Kits:*

TransIT[®]-In Vivo Gene Delivery System (Product # MIR 5100)

RNA Interference Products:*

TransIT-TKO[®] siRNA Transfection Reagent (Product # MIR 2150)

TransIT[®]-siQUEST[™] siRNA Transfection Reagent (Product # MIR 2110)

siXpress[®] PCR Vector Systems (Product # MIR 7300, 7301, 7302)

TransIT-TKO[®] HTS-96 Plates (Product # MIR 2530, 2540, 2550, 2560, 2570)

*These products are available in additional sizes.

The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.

Mirus Transfection Reagents are covered by United States Patent No. 5,744,335; 5,965,434; 6,180,784; 6,383,811, 6,593,465 and patents pending.

This product is sold to the Buyer with a limited license to use this product for research only. This product, or parts from this product, may not be re-packaged or re-sold without written permission from Mirus Bio Corporation.

TransIT, TransIT-TKO, TransIT-Neural, HeLaMONSTER, MiraCLEAN, siXpress and Label IT are registered trademarks of Mirus Bio Corporation.

Label IT Tracker and siQUEST are trademarks of Mirus Bio Corporation.

© 1997-2008, Mirus Bio Corporation. All rights reserved.