For life science research only.
Not for use in diagnostic procedures.

β-Gal Staining Set

Version: 06
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For the histochemical staining of cells and tissue sections demonstrating β-galactosidase activity

Cat. No. 11 828 673 001 1 set
100 tests in 3.5 cm dishes

Store the set at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	red	β-Gal Staining Set, X-Gal solution	▪ Stock solution in dimethyl sulfoxide (DMSO). ▪ Ready-to-use stabilized formulation.	1 bottle, 6 ml
2	blue	β-Gal Staining Set, Iron buffer	▪ Potassium ferrocyanide and potassium ferricyanide in phosphate buffered saline (PBS). ▪ Ready-to-use stabilized formulation.	1 bottle, 100 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the set is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	red	X-Gal solution	Store at +2 to +8°C. ⚠ ***DMSO-containing solutions may solidify upon storage at +2 to +8°C. If this occurs, incubate vial at +37C in a water bath and vortex until solution appears clear.***
2	blue	Iron buffer	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- 6- and 24-well plates
- 6 and 10 cm dishes
- Regular and light microscope
- Humidified incubator

For preparation of working solutions

- 37% formaldehyde
- 25% glutaraldehyde
- PBS (phosphate buffered saline)*

For staining protocols

- DOTAP Liposomal Transfection Reagent*
- PBS*
- Antibodies conjugated with β-galactosidase
- Triton X-100*
- Mounting medium, such as 70% glycerol in PBS (optional)

1.4. Application

The β-Gal Staining Set is used to detect:
- Bacterial β-Gal activity for direct visualization in transfected cells.
- β-galactosidase conjugated to antibodies in immunohistochemical procedures.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The β-Gal Staining Set can be used with cells transfected with a β-Galactosidase-encoding construct and tissue sections.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Preparation of staining solution

1. Examine the X-Gal solution; it should be clear before use.
   – DMSO-containing solutions may solidify upon storage at +2 to +8°C. If this occurs, incubate Bottle 1 at +37°C in a water bath and vortex until solution appears clear.

2. Dilute 1 part of X-Gal solution with 19 parts of Iron buffer (Bottle 2) and mix thoroughly for 10 minutes.

3. Prepare fresh before use. Do not store.

Required volumes of staining solutions per well or dish

Solution	24-well plate [µl]	6-well plate (3.5 cm dish) [µl]	6 cm dish [µl]	10 cm dish [µl]
X-Gal solution	25	50	100	150
Iron buffer	475	950	1,900	2,850
Total Volume [ml]	**0.5**	**1**	**2**	**3**

Preparation of fixative

Fixative: 2% formaldehyde, 0.2% glutaraldehyde in PBS.

1. To prepare 10 ml of Fixative, mix 540 µl formaldehyde (37%) with 80 µl glutaraldehyde (25%).
2. Add 9.38 ml PBS.
3. Prepare fresh before use.

Required volumes of fixative per well or dish

Solution	24-well plate [ml]	6-well plate (3.5 cm dish) [ml]	6 cm dish [ml]	10 cm dish [ml]
Fixative	0.5/well	1 – 2/well	2 – 4/dish	3 – 5/dish

2.2. Protocols

Staining transfected cells

The following protocol is for 3.5 cm culture dishes (6-well plate).

1. Transfect cells, for example, using DOTAP Liposomal Transfection Reagent* with a β-galactosidase-encoding construct, as described in the respective Instructions for Use.
2. Wash cells once with PBS.
3. Remove PBS and add 1 to 2 ml Fixative per well of a 6-well culture plate.
   – Incubate for 15 minutes at +15 to +25°C.
4. Remove Fixative and wash cells 3 times with PBS.
   If required, the fixed and washed cells may be stored at +2 to +8°C in PBS for up to 1 week before staining.
5. Add 1 ml Staining solution per well of a 6-well culture plate, and incubate for 0.5 to 3 hours at +37°C until cells are stained blue.
   – Check for color development under a microscope and continue incubation if necessary.
   – When analyzing highly confluent cells, monitor incubation time closely. Staining for too long may result in false-positive results, with blue precipitation occurring on cells adjacent to a positive cell.
   – To prevent dishes from drying out during prolonged storage, use a humidified incubator, or place the dishes into a moist chamber.
6. Remove Staining solution and wash cells 3 times with PBS.
7. Analyze in PBS under light microscopy without phase-contrast.
   – To determine transfection efficiency, count stained and non-stained cells in an adequate number of random viewing fields on the plate; calculate percentage of stained cells in the total cell population.
8. For long-term storage, replace PBS with mounting medium.

Immunohistocytochemistry staining

1. Process specimen for immunohistochemistry according to standard protocols.

2. Incubate specimen with antibodies conjugated with β-galactosidase.

3. Wash at least 5 minutes in PBS.
   – Add 0.02% Triton X-100 for optimal reduction of nonspecific binding of antibodies.

4. Cover tissue with a sufficient volume of Staining solution to fully cover the specimen, approximately 50 µl.
   – Incubate for 15 to 30 minutes at +37°C in a humid chamber until cells are stained blue.
   – Check for color development under a microscope and continue incubation time if necessary.

5. Wash specimen 3 times with PBS.

6. Analyze in PBS under light microscopy.

7. For long-term storage, replace PBS and mount in glycerol before storage.

2.3. Parameters

Specificity

The β-Gal Staining Set allows specific detection of bacterial *lacZ* gene encoded β-galactosidase.

3. Additional Information on this Product

3.1. Test Principle

How this product works

The *lacZ* gene of *E. coli*, encoding for β-galactosidase β-Gal, is used extensively as a reporter of gene expression in a variety of systems, including:

- Bacteria and yeast, mammalian, avian, and insect cells.
- Whole insects and nematodes.

The enzymatic activity of bacterial β-Gal can be assayed readily from transfected cells and tissue. It exhibits maximal enzymatic activity at neutral to alkaline pH, with an optimum pH of 7.0 to 7.5, which makes discrimination from endogenous mammalian β-galactosidase (optimum pH 3.0 to 6.0) possible.

β-Gal has become a preferred reporter for normalization in co-transfection experiments, especially due to the colorimetric substrates ONPG and CPRG*, and their ease of use for determining β-Gal activity in cell extracts. The availability of methods for highly sensitive detection of β-galactosidase by chemiluminescent reporter gene assays* and a standardized β-Gal ELISA* have led to an even more widespread use of β-Gal reporter constructs.

Another advantage of the β-Gal system is the availability of the precipitating β-Gal substrate 3-indolyl-β-D-galactopyranoside (X-Gal*), classically used in prokaryotic clone selection procedures. In combination with a specific iron buffer, X-Gal offers an easy-to-use histochemical procedure, enabling detection of individual cells expressing a transfected bacterial *lacZ* gene by light microscopy. It thereby yields a blue stain within positively transfected cells without producing background. Histochemical staining is useful for determining the percentage of transfected cells, and specifically to optimize transfection conditions. It may also be used for staining of tissue sections, such as evaluating *in vivo* transfection efficiencies in animal model systems.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
β-Gal Reporter Gene Assay, chemiluminescent	1 kit, 500 assays (microplate format) 250 assays (tube format)	11 758 241 001
DOTAP Liposomal Transfection Reagent	2 ml, 5 x 400 µl, 5 x 400 µg	11 202 375 001
X-Gal	100 mg, *Not available in US*	11 680 293 001
	100 mg	03 117 073 001
	250 mg	10 651 745 001
	1 g	10 745 740 001
	2.5 g	10 703 729 001
CPRG	250 mg	10 884 308 001
Triton X-100	100 ml	10 789 704 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany