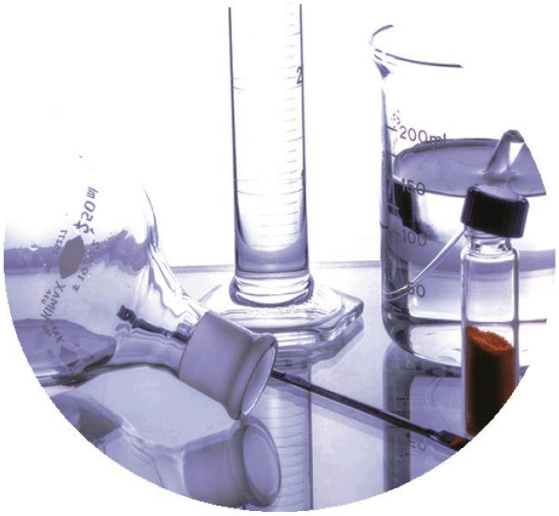

Assay kits

X-Gal Staining Kit

X-Gal Staining kit

Protocol

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IMPORTANT NOTES – Before you begin

Ready-to-use assay system for determining the transfection efficiency based on in situ β-galactosidase staining

- ✓ Lac Z is one of the most frequently used reporter gene in transfection experiments because the gene product specific properties. Indeed, the Lac Z encoded protein, β-galactosidase, is very stable, resistant to proteolytic degradation and easily tested.
- ✓ All the necessary reagents provided in this assay kit offer a rapid, simple and sensitive method to determine the percentage of Lac Z transfected cells. Indeed, β-Galactosidase catalyzes the hydrolysis of β-Galactosides (i.e. X-Gal) into blue precipitates. Consequently, cells transfected with β-galactosidase expressing plasmid appear blue following fixation and incubation with X-Gal substrate. Blue cells can be visualized by microscopy.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

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Any questions?

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X-Gal Staining Kit | Specifications

Package content	GX10003 Fixing Buffer (125 ml) 10X PBS (75 ml) Staining Buffer (125 ml) 25X Stock Solution of X-Gal (4 X 1 ml) The kit contains sufficient reagents to perform 50 assays of 60 mm dishes.
Shipping conditions	The kit is shipped with gel pack (4°C).
Storage conditions	Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions Fixing Buffer (+4°C) 10X PBS (+4°C) Staining Buffer (+4°C) 25X Stock Solution of X-Gal (-20°C)
Shelf life	1 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

1. Usage

1. Transfect cells with a plasmid expressing Lac Z gene
2. Fix the cells with the fixing buffer
3. Stain the cells with X-gal staining solution
4. Observe the cells with blue stain under a microscope
5. Calculate the percentage of blue cells in the total population. Controls such as non-transfected cells or cells transfected with a blank plasmid (mock-transfected cells) must be used to verify the level of background activity caused by endogenous β-galactosidase

2. General Considerations

- Before use, dilute the 10X PBS to 1X with deionized water. The surplus of unused 1X PBS may be stored at +4°C or room temperature for future use.
- Dilute the 25X stock solution of X-Gal to 1X with Staining Buffer. The 1X solution of X-Gal must be prepared freshly each time the assay is performed. Discard the surplus of X-Gal solution excess.

3. General Protocol

1. 24-72 hours after transfection aspirate the culture medium from the dish.
2. Wash the cells once with 1X PBS.
3. Add Fixing Buffer to the dish and incubate for 10-15 minutes at room temperature.
   CAUTION: The Fixing Buffer contains chemicals that are corrosive, carcinogenic and poisonous and must be handled carefully (see Materials Safety Data Sheet for further details). Wear gloves, goggles, lab coats and other protective gear when handling the Fixing Buffer. Some products are harmful if inhaled, swallowed or absorbed through the skin.
4. Discard the fixing solution from the culture dish and carefully wash the cells 2 times with 1X PBS.
5. Add freshly prepared the 1X staining solution of X-Gal to the dish. Incubate the cells between 1 to 18 hours at +37°C in a humidified incubator. The incubation time should be adjusted according to the transfection efficiency.
6. Remove the X-Gal staining solution and wash the cells once with 1X PBS.
7. Add 1X PBS to the dish and proceed to the examination of the blue cells under a light microscope; count the stained and unstained cells in randomly selected fields. Calculate the percentage of stained cells in the total population.
8. If you need to store the plates for several weeks or months, fix each well with 1ml of 10% formalin in PBS (not supplied) for 10 minutes at room temperature, rinse with 1X PBS and store in 1X PBS or 70% glycerol solution (not supplied) at +4°C

Volumes of solution recommended for various culture dishes are listed in the subsequent table.

Type of culture dish	Fixing Buffer (µl/well)	Staining Buffer (µl/well)	1X PBS Washing Buffer (µl/well/wash)
Chambered slide	500	500	1000
24-well plate	250	250	500
12-well plate	500	500	1000
6-well plate	1000	1000	2000
60 mm dish	2500	2500	3000
100 mm dish	5000	5000	8000

NOTES

Additional products

- **ONPG assay kit** to quantify high expression level of beta-Gal. Broad Spectrum DNA transfection reagent
- **CPRG assay kit** to quantify low expression level of beta-Galactosidase

Purchaser Notification

Limited License
The purchase of the X-Gal Staining Kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the X-Gal Staining Kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all X-Gal Staining Kit reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a X-Gal Staining Kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the X-Gal Staining Kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations
X-Gal Staining Kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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