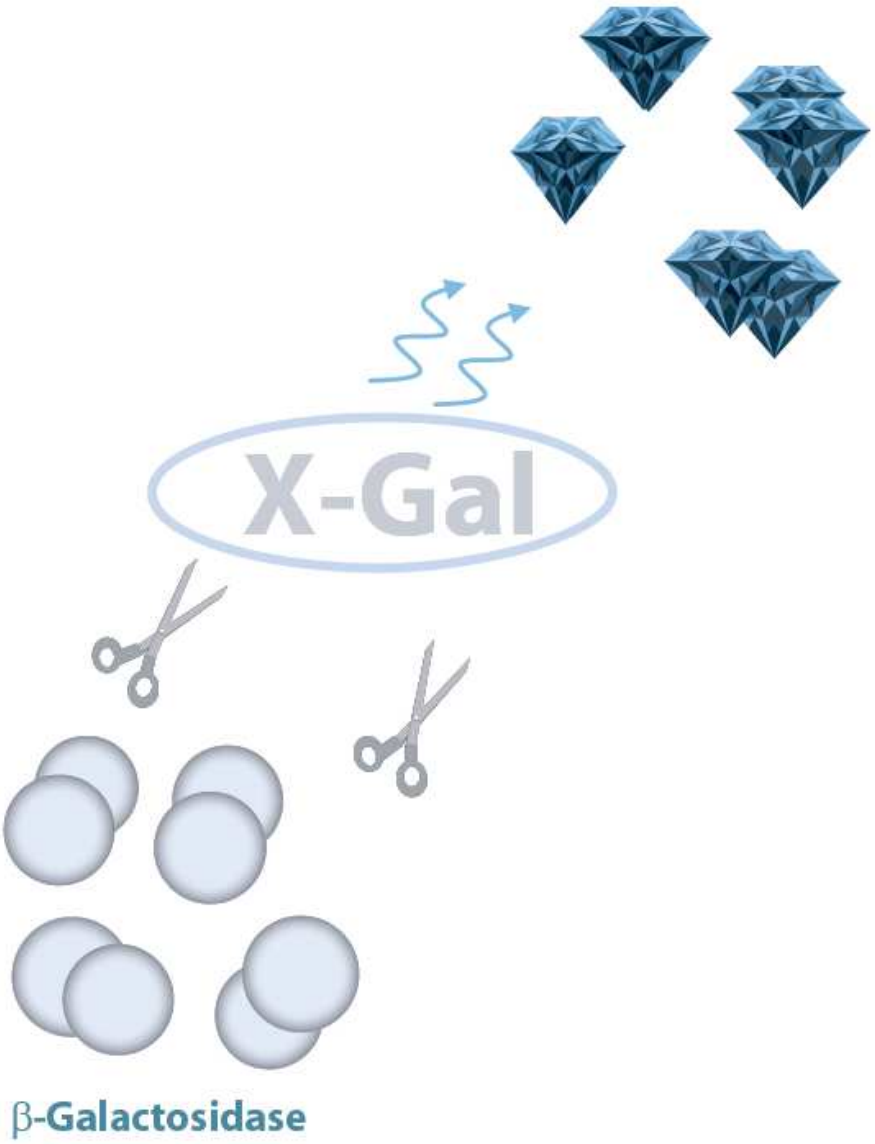
X-Gal staining Kit

INSTRUCTION MANUAL



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X-Gal Staining Kit

Instruction Manual

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

Catalog Number: GX10003

You can order this product by contacting us. For all other additional information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09 - FRANCE
Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515
contact@ozbiosciences.com
order@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117 - USA
Ph : + 1-858-246-7840
Fax : + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com

www.ozbiosciences.com

1. Technology

1.1. Description

Congratulations on your purchase of the X-Gal Staining Kit!

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.

This X-Gal Staining Kit is:

- Simple and Rapid
- Ready-to-use
- Economical

1.2. Kit Contents

The kit contains sufficient reagents to perform 50 assays of 60 mm dishes.

Component	Quantity	Storage
Fixing Buffer	125 ml	4°C
10X PBS	75 ml	4°C
Staining Buffer	125 ml	4°C
25X Stock Solution of X-Gal (5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside)	4 X 1 ml	-20°C

Stability and Storage

Storage — Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions (see table above). Kit's components are stable for at least 1 year at the recommended storage temperature.

Shipping condition — The X-Gal Staining kit is shipped with gel pack (4°C)

2. Applications and Protocols

2.1. 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

2.2. 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.

2.3. 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

3. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
SilenceMag *(for siRNA application)*
NeuroMag *(dedicated for neurons)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for retrovirus and Lentivirus)*
AdenoMag *(for Adeno viruses)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
Ecotransfect *(Economical reagent for routine transfection)*
FlyFectin *(for Insect cells)*
VeroFect *(for Vero cells)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)*
3DfectIN *(for hydrogels culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ 25µg pVectOZ-SEAP 25µg
ASSAY KITS
Bradford – Protein Assay Kit β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g G-418, Sulfate 1g X-Gal powder 1g

Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list. http://www.ozbiosciences.com

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Director of Business Development
OZ Biosciences SAS
Parc Scientifique et Technologique de Luminy
Bâtiment Grand Luminy technopole
Case 922 zone entreprise
13288 Marseille Cedex 9, France
Ph: +33 (0)4.86.94.85.16
Fax: +33 (0)4.86.94.85.15
E-mail: contact@ozbiosciences.com

CONTACTS

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
FRANCE

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117
USA

Ph : + 1-858-246-7840
Fax : + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

www.ozbiosciences.com

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