

Transfection tools

X-GAL Substrate

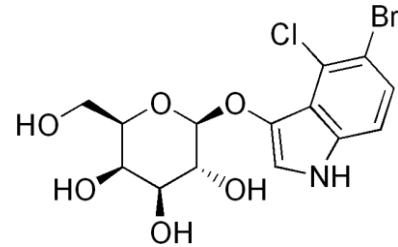
Protocol

IMPORTANT NOTES – Before you begin

✓ **X-Gal** is a synthetic β -Galactoside that is hydrolyzed by the β -Galactosidase enzyme into blue precipitates. Consequently, bacteria, cells, tissues or organisms expressing β -galactosidase appear blue following fixation and incubation with X-Gal substrate. Blue cells can be visualized by microscopy.

✓ **Main Features are:**

- High purity > 99%
- Good solubility and great sensitivity
- Perfect for cells, tissues and organism staining
- Easy to use



✓ **X-Gal:**

- *Chemical Name:* 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside
- *Molecular Formula:* C₁₄H₁₅BrClNO₆
- *Molecular weight:* 408.63 g/mol
- *CAS Number:* 7240-90-6
- *Molecular biology grade and premium pure.*

CAUTION: X-Gal is sensitive to strong oxidizing agent, moisture and light.

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

➔ www.ozbiosciences.com

Any questions?

➔ tech@ozbiosciences.com

X-Gal Substrate | Specifications

Package content	XG31000 X-Gal Substrate (1g)
Shipping conditions	The X-Gal is shipped at room temperature
Storage conditions	Upon receipt and for long-term use, store the powder in a tightly closed and desiccated container at -20°C. We recommend warming the desiccator to room temperature for at least one hour prior to removing the X-Gal. X-Gal is hygroscopic and it is critical to avoid any condensation of water on the X-Gal crystals.
Shelf life	2 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

Applications

- Cells, tissues and organisms staining
- Lac Z reporter gene assays
- Transfection/transduction with Lac Z reporter gene and Lac Z-fusion constructs
- Blue/white colony screening assay, discern recombinant (white) from non-recombinant (blue) colonies
- Detection of β -galactosidase activity in immunological and histochemical procedures.

The instructions given below represent successfully applied protocols. They can be used as guidelines to quickly achieve very good staining. Optimal conditions do vary according to cell cultures, bacteria, tissues and assay sensitivity and the final quantity of X-Gal might have to be adjusted to achieve best results.

1. Preparation X-Gal stock solution

1. Dissolve 100 mg of X-Gal into 4 or 5mL of dimethylformamide (DMF).
2. Divide into 1mL aliquots (preferably use amber vials).
3. Store X-Gal in the dark. You can either use amber vials or wrap tubes in aluminum foil.
4. Store at -20°C

NB: Solution does not need to be filtered

2. Preparation X-Gal for Prokaryotic cells

1. Prepare LB/agar or similar media by standard methods.
2. After autoclaving allow the bottle containing the agar to cool for 20 min. and then place it into a water bath at 48°C.
3. Allow the agar to equilibrate to the 48°C temperature for an appropriate period of time, which is dependent on the volume of the solution.
4. Add 1/100th volume of the 2% X-gal in DMF and gently swirl the media.
5. Add other components such as antibiotic or IPTG to the agar media.
6. Pour the agar into petri dishes and allow to solidify for at least 20 min. at room temperature.
7. After plating your bacteria, incubate at 37°C overnight until blue color develops.

3. Preparation X-Gal for Eukaryotic cells and tissues

There are many protocols used for X-Gal with eukaryotic cells, tissues and organisms. If you don't have a favorite protocol we recommend that you visit the following web sites:

<http://www.cshprotocols.org/>

<http://www.protocol-online.org/>

<http://www.scientistsolutions.com/Science-Forum.aspx>

In addition, you may find an appropriate protocol for your needs in "Current Protocols in Molecular Biology".

Additional products

- **DMNPE-caged luciferin**; a good alternative to D-Luciferin, used to measure luciferase activity in live cells.
- **D-Luciferin K+ and Na+** for *in vitro* and *in vivo* bioluminescent assays

Purchaser Notification

Limited License

The purchase of the X-Gal Substrate 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 Substrate. 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 Substrate 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 Substrate 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 Substrate 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 Substrate 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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