

# EZ-BLUEZ™

## Ready2USE™ X-Gal Solution

**5-Bromo-4-Chlor-3-Indolyl-b-D-Galactopyranoside.**

**Concentration: 10 mg/mL**

**Catalog#: MESL-2220**

**For research use only. Not for use in diagnostic procedures.**

**Storage:** Store at 4°C

**Stability:** Refer to the product label

**Toxicity:** EZ-BLUEZ does not contain DMF or DMSO.

**Quality control:** Each batch is tested against classical X-gal/IPTG method, with 2 different strains expressing pUC19 (high copy plasmid) and BAC2 (low copy plasmid). EZ-BLUEZ is prepared at 10 mg/mL concentration for easy dilution.

**For detection of B-galactosidase activity in bacterial colonies:**

**For preparation of LB agar plates**

It is convenient to add EZ-BLUEZ directly to the molten LB Agar (at 45°C maximum).

- Use 4 µl of EZ-BLUEZ solution per mL of medium (for 500 mL of LB Agar, add 2000 µl).
- Add selection antibiotic according to your standard protocol.
- Mix well and pour plates for use on poured LB plates.
- Remove LB plate from the fridge.
- Let the plate warm at room temperature and use as needed.

**For use with pre-poured agar plates:**

- Spread 100 µl of EZ-BLUEZ across the surface using a suitable spreader (for plates containing 25 mL of medium).
- Let the plate dry before use.

**For X-Gal staining of mammalian cells expressing the LacZ gene:**

1. Discard the medium from the cell culture dishes or plates and wash once with PBS previously equilibrated at 37°C.
2. Fix the cells during 10 minutes with a PBS solution containing 0,2% glutaraldehyde and 2% formaldehyde. Add enough fixative solution to completely cover the cells.
3. Discard the fixative solution and rinse cells thoroughly three times in PBS at room temperature (remove the first rinse immediately, leave the second and third rinses on the cells for 5 minutes).
4. Add a minimal amount of staining solution containing 1 mg/mL of X-Gal (see composition below).
5. Incubate for 1 hour to overnight at 37°C.

**Solutions:**

1 L PBS: 144 mg KH<sub>2</sub>PO<sub>4</sub>

9 g NaCl

795 mg Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O

**Staining solution: Prepare 250 mL of PBS containing**

2 mM Magnesium Chloride

5 mM K<sub>3</sub>Fe(CN)<sub>6</sub>

5 mM K<sub>4</sub>Fe(CN)<sub>6</sub>·3H<sub>2</sub>O

25 mL EZ-BLUEZ