Protocol: Preparation and Use of X-Gal

1. Preparation of X-Gal Stock Solution

Materials

- X-Gal powder
- DMSO or DMF (anhydrous)
- Light-protected tubes (amber or foil-wrapped)

Procedure

1. Allow X-Gal powder to equilibrate to room temperature before opening.
2. Dissolve X-Gal at **20 mg/mL** in DMSO or DMF
   (e.g. 100 mg X-Gal in 5 mL solvent).
3. Mix gently until fully dissolved. Avoid prolonged light exposure.
4. Aliquot into small volumes (e.g. 100–500 µL).
5. Store aliquots at **–20 °C**, protected from light.

Stock solution is typically stable for ~12 months when aliquoted and stored properly.

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2. Preparation of X-Gal Agar Plates

Typical final concentration

- **40 µg/mL X-Gal**
- **0.1–1 mM IPTG** (commonly 0.5 mM)

Procedure

1. Prepare LB agar containing the appropriate antibiotic.
2. Cool molten agar to ~50–55 °C.
3. Add X-Gal stock to the desired final concentration.
4. Add IPTG (from sterile stock).
5. Mix gently, pour plates, and allow to solidify in the dark.
6. Store plates at **4 °C**, protected from light (use within 1–2 weeks).

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3. Use in Blue–White Screening

1. Transform competent *E. coli* cells with plasmid DNA.
2. Plate cells onto LB agar plates containing antibiotic, IPTG, and X-Gal.
3. Incubate at **37 °C for 12–18 hours**.

4. Score colonies:
    - **Blue**: functional lacZ (no insert)
    - **White**: disrupted lacZ (insert present)

Notes & Tips

- Always protect X-Gal (solid and solution) from light.
- Do not autoclave X-Gal.
- Avoid repeated freeze–thaw of stock solutions.