Blue/White Screening of Bacterial Colonies X-Gal/IPTG Plates

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Abstract

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Guidelines

Dry X-Gal/IPTG-coated media in a laminar flow hood for approximately 30 minutes before use.

Plate Surface
1. Dry media plates in a laminar flow hood.
2. Add 40 µl 100mM IPTG and 120 µl X-Gal (20 mg/ml) to the surface of each plate and spread over entire surface.
Note: The edges of the plate are difficult to spread adequately and may give false positives. We advise picking colonies in the middle of the plate, if possible, for best results.

Before start

Prepare 20 mg/ml X-Gal solution in DMF (See X-Gal Stock Solution Procedure). For reduced DMF toxicity in media, you can alternatively make a 100 mg/ml X-Gal solution in DMF (this concentration is only stable at -20°C for ~1 week).

Prepare 100mM IPTG solution in dH2O (or dilute from 1M IPTG Stock Solution).

Materials

IPTG GB-I2481C by P212121

X-Gal GB-X4281C by P212121

Protocol

Step 1.
Cool autoclaved growth media agar to 50°C.

Step 2.
Add 10 µl X-Gal Solution (20 mg/ml) per 1 mL of Media
(or 2 µl X-Gal Solution (100 mg/ml) per 1 mL of Media).

DURATION
00:02:00

Step 3.
Add 10 µl IPTG (100mM) per 1 mL of Media for a final concentration of 1mM.

REAGENTS
IPTG GB-I2481C by P212121

DURATION
00:03:00

Step 4.
Add screening antibiotic of choice (Ampicillin, Kanamycin, Carbenicillin, etc).

REAGENTS
Ampicillin Sodium Salt TWA-A-301 by P212121

DURATION
00:03:00

Step 5.
Pour plates and allow to cool to room temperature (usually at least 30 minutes) before use.

DURATION
00:40:00

Step 6.
Spread transformed competent cells as desired.

Note: Blue/White Selection plates are generally stable for only 1 week if stored at 4°C in clear sleeves, but may be stored in the dark (or a dark sleeve) at 4°C for up to 1 month.

DURATION
00:05:00