Nov 04, 2014

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

DOI
dx.doi.org/10.17504/protocols.io.cjauid

Sean Seaver
P212121

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.cjauid

External link: http://store.p212121.com/x-gal/

Protocol Citation: Sean Seaver: Blue/White Screening of Bacterial Colonies X-Gal/IPTG Plates. **protocols.io** https://dx.doi.org/10.17504/protocols.io.cjauid

Protocol status: Working

Created: October 23, 2014

Last Modified: March 26, 2018

Protocol Integer ID: 322

Abstract

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

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.

Materials

MATERIALS

IPTG **P212121 Catalog #**GB-I2481C

X-Gal **P212121 Catalog #**GB-X4281C

STEP MATERIALS

IPTG **P212121 Catalog #**GB-I2481C

Ampicillin Sodium Salt **P212121 Catalog #**TWA-A-301

IPTG **P212121 Catalog #**GB-I2481C

Ampicillin Sodium Salt **P212121 Catalog #**TWA-A-301

Protocol materials

IPTG **P212121 Catalog #**GB-I2481C In Materials, Materials, Materials, Step 3

Ampicillin Sodium Salt **P212121 Catalog #**TWA-A-301 In Materials, Materials, Step 4

X-Gal **P212121 Catalog #**GB-X4281C Materials

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).

1 Cool autoclaved growth media agar to 50°C.

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).
00:02:00

3 Add 10 µl IPTG (100mM) per 1 mL of Media for a final concentration of 1mM.
00:03:00
IPTG **P212121 Catalog #**GB-I2481C

4 Add screening antibiotic of choice (Ampicillin, Kanamycin, Carbenicillin, etc).
00:03:00
Ampicillin Sodium Salt **P212121 Catalog #**TWA-A-301

5 Pour plates and allow to cool to room temperature (usually at least 30 minutes) before use.
00:40:00

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.
00:05:00