

Media Preparation:

Making agar plates containing Ampicillin, X-gal, and IPTG

You will need to make up the following stocks, as well as L-Broth and L-Agar

3% X-gal in DMF:

0.15g in 5 ml DMF (dimethyl formamide) Store at -20 degrees C.

100 mM IPTG:

.12g in 5ml sterile distilled water. Dissolve and filter sterilize.
Store in aliquots at - 20 degrees C.

100 mg/ml Ampicillin

.5 g in 5 ml sterile distilled water. Dissolve and filter sterilize. Store in aliquots at -20.

L-Broth

5 g Tryptone

2.5 g Yeast extract

2.5 g NaCl

Distilled water to 500 ml

Dissolve solids in the water, then divide the L-broth into 100 ml aliquots.

Autoclave for 30-45 minutes.

NB: LB and L-agar MUST be autoclaved on the same day they are made up. Make sure you put autoclave tape on the bottles before autoclaving.

L-Agar

5g Tryptone

2.5 g Yeast extract

2.5 g NaCl

1.5 % Agar (7.5g in 500 ml)

Distilled water to 500 ml.

The agar will not dissolve until autoclaved. Autoclave for 30-45 minutes.

1. When the agar has been autoclaved, allow it to cool until the bottle can be held with bare hands.
2. Add 1 ml (per litre of L-Agar) each of the Amp, IPTG, and X-Gal stocks (e.g. 500 microliter each in 500 ml L-agar.)
3. Invert to mix thoroughly.
4. Carefully pour out into sterile plastic petri dishes.
5. Allow to set, then store wrapped in plastic in the cold room. Remember to label them as AIX plates, with date and your initials.

To make Amp plates, follow the same procedure but add only Amp to the L-Agar.
(These instructions courtesy of Jon Widom's Lab.)

