

Xgal addition to KB agar

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Outline

Xgal need to be added to KB agar before plating to be able to characterise *lacZ* mutants of *Pseudomonas fluorescens* and other bacterial species which turn blue on the plates.

Xgal is dissolved in DMSO before addition to the agar. DMSO is super toxic so this needs to be done under the fume hood.

Concentration

300 mg of Xgal is added to 15 mL of DMSO. 4.5 mL of this solution is added to 1 L of agar.

Some common concentrations:

- 1 L of agar : 100 mg Xgal in 5 mL DMSO.
- 2 L of agar : 200 mg Xgal in 10 mL DMSO.
- 3 L of agar : 300 mg Xgal in 15 mL DMSO.

Method

- Get the Xgal out of the AB freezer (top shelf is the communal shelf).
- Put foil round a 50 mL falcon tube (Xgal is light sensitive).
- Measure out the required amount of Xgal on the sensitive scales and place it in the falcon tube.
- Get the DMSO from the chemicals cabinet (underneath the fume hood) and add the desired amount to the falcon tube using a serological pipette.
- Add this to agar (not too hot).
- Shake agar
- Plate