TOKU-E Ampicillin Preparation and Selection Guide

Background

Ampicillin is a β-lactam antibiotic routinely used in bacterial selection procedures to select for bacteria (usually *E. coli*) that have been transformed with an ampicillin resistance plasmid (pUC19, etc.). Ampicillin resistance is usually due to production of β -lactamase enzymes which cleave the β-lactam ring rendering the antibiotic inactive.

The information below will outline preparation, storage, and a general selection procedure for Ampicillin-resistant bacteria.

Preparation and storage

Ampicillin (TOKU-E item # A043) is packaged and shipped in powder form but can be dissolved at a 100 mg/mL stock solution. Ampicillin is frequently used in LB plates or broth for selection procedures at a concentration of 100 µg/ml.

Stock solution

An Ampicillin stock solution can be prepared at a concentration of 100 mg/mL and should be stored at -20ºC.

1. Add 1 g (1000 mg) of Ampicillin to 10 mL of dH_2O
2. Sterilize the solution using a 0.22 µm filter
3. Store solution in different aliquots at -20ºC

LB-Ampicillin agar preparation:

1. Dissolve the following in 500 mL dH_2O:

5.0 g tryptone
2.5 g yeast extract
5.0 g NaCl
7.5 g agar
25 mg Ampicillin
or:

20 g pre-mixed LB agar powder
25 mg Ampicillin

2. Boil solution on stirring hot plate for 1 – 2 min.

3. Autoclave for 20 minutes and let cool to 50-60ºC.

4. Pour approximately 10 mL of molten LB agar into each plate.

5. Allow plates to solidify for approx. 20 min.

Preparation and storage

1. Using a sterile loop, take a sample of suspected Ampicillin resistant bacteria from a colony or broth suspension and streak for isolation (using preferred method) on LB-Ampicillin plates.

2. Incubate plates inverted overnight (24 hrs.) at 37ºC.

3. Any resulting colonies should represent Ampicillin-resistant isolates.

References

1) Cold Spring Harbor Protocols (2011). Agar plates with LB medium and ampicillin (50 ug/ml) (Link)

2.) Erlangen F (2009) Preparing antibiotics stock solution and ampicillin agar plates. Protocol-online. (Link)