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, others). Ampicillin resistance is usually due to production of beta-lactamase enzymes which cleave the beta-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 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 -20^{o}C.

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 -20^{o}C

LB-ampicillin agar preparation:

1. Dissolve the following in 500 mL dH_2O:

a) 5g tryptone
b) 2.5 g yeast extract
c) 5.0 g NaCl
d) 7.5 g agar
e) 25 mg ampicillin

Or

a) 20 g pre-mixed LB agar powder
b) 25 mg ampicillin

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

3. Autoclave for 20 minutes and let cool to 50-60^{o}C.

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

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

Selection of ampicillin resistant bacteria:

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 37^{o}C.

3. Any resulting colonies should represent ampicillin resistant isolates.

References:

1.) "Agar Plates with LB Medium and Ampicillin." *CSH Protocols*. Cold Spring Harbor, n.d. Web. 1 Nov. 2013.

2.) Erlangen, FAU. "Preparing Antibiotics Stock Solution and Ampicillin Agar Plates." *Protocol-online*. N.p., n.d. Web. 1 Nov. 2013.

3.) http://cell-lines.toku-e.com/