

Selection of Ampicillin Resistant Bacteria

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Abstract

[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.

Citation: Sean Seaver Selection of Ampicillin Resistant Bacteria. **protocols.io**

dx.doi.org/10.17504/protocols.io.cixufm

Published: 01 Nov 2014

Materials

 Ampicillin Sodium Salt [TWA-A-301](#) by [P212121](#)

Protocol

Step 1.

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

Add 1 g (1000 mg) of ampicillin to 10 mL of dH₂O



REAGENTS



Ampicillin Sodium Salt [TWA-A-301](#) by [P212121](#)



DURATION

00:03:00

Step 2.

Sterilize the solution using a [0.22 \$\mu\$ m filter](#)

Store solution in different aliquots at -20°C



REAGENTS



Syringe Filter [LI-PES-0/22-13-S](#) by [P212121](#)



DURATION

00:02:00

Step 3.

LB-ampicillin agar preparation:

Dissolve the following in 500 mL dH₂O:

5g 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 (or LB agar capsules)
25 mg ampicillin



REAGENTS



LB Miller Agar Capsules [RP](#) by [P212121](#)



DURATION

00:15:00

Step 4.

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



REAGENTS



Hot plate and stirrer [BM-H4000](#) by [P212121](#)



DURATION

00:02:00

Step 5.

Autoclave for 20 minutes and let cool to 50-60°C.



DURATION

00:20:00

Step 6.

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



REAGENTS



Petri Dish [LI-PD01100](#) by [P212121](#)



DURATION

00:03:00

Step 7.

Allow plates to solidify for approx. 20 min.



DURATION

00:20:00

Step 8.

Selection of ampicillin resistant bacteria:

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.



REAGENTS



Inoculating loop [EM-65](#) by [P212121](#)



DURATION

00:02:00

Step 9.

Incubate plates inverted overnight (24 hrs.) at 37°C.

 DURATION

24:00:00

Step 10.

Any resulting colonies should represent ampicillin resistant isolates.