

Antibiotic Concentrations for Bacterial Selection (modified Addgene; includes L-arabinose instructions)

Background Information: Plasmids carry antibiotic resistance genes, which confer antibiotic resistance to the bacteria carrying them. The presence of these antibiotic resistance genes on plasmids allows researchers to easily isolate bacteria containing the plasmids from those that do not by artificial selection (i.e. growing them on or in a medium containing the antibiotic). You should check your plasmid's datasheet or the plasmid map to determine which antibiotic to add to your LB media or LB agar plates.

Preparing Antibiotics (arabinose is at the end of this protocol)

1. Create a stock solution of your antibiotic. Addgene recommends making 1000X stock solutions and storing aliquots at -20°C. **Note:** Stock solutions must be filter sterilized (see below).

Antibiotic Concentrations:

Antibiotic	Recommended Stock Concentration	Recommended Working Concentration
Ampicillin	100 mg/mL	100 µg/mL
Bleocin	5 mg/mL	5 µg/mL
Carbenicillin*	100 mg/mL	100 µg/mL
Chloramphenicol	25 mg/mL (dissolve in EtOH)	25 µg/mL
Coumermycin	25 mg/mL (dissolve in DMSO)	25 µg/mL
Gentamycin	10 mg/mL	10 µg/mL
Kanamycin	50 mg/mL	50 µg/mL
Spectinomycin	50 mg/mL	50 µg/mL
Tetracycline	10 mg/mL	10 µg/mL

Note: Unless otherwise indicated, the antibiotic powder can be dissolved in dH₂O and filter sterilized.

2. To use, dilute your filter sterilized antibiotic into your LB medium at 1:1,000. For example, to make 100 mL of LB/ampicillin growth media, add 100 µL of a 100 mg/mL ampicillin stock (1000X stock) to 100 mL of LB.
3. To make media containing L-arabinose (an inducer for *araBAD* operon), you will need to make a 100X filter sterilized solution by dissolving 200 mg of arabinose into 1 mL of sterile dH₂O.
4. To filter sterilize the stock solutions of additives, you will dissolve the additive into the required amount of sterile water. For example to make stock ampicillin you would dissolve 100 mg into 1 mL of sterile water and mix thoroughly. Next, you would push the solution through a 0.22 micron polytetrafluoroethylene (PTFE), cellulose acetate, or polyethersulfone membrane fiber filter. Please note that the syringe, filter, and filter housing must all be autoclaved and sterile prior to filtration. The sterile additive should be filtered into a sterile 1.5 mL microcentrifuge tube, labeled, and stored at -20 C. The link at the bottom of this document may be used to reorder filters.

<https://us.vwr.com/store/product/4830563/vwr-syringe-filters>