

Antibiotic	Stock solution (mg/ml)	Solvent****	Storage temp (°C)	Working concentration		Function
				µg/ml	dilution	
Ampicillin*	50	H ₂ O	-20° C	50	1.0 µl/ml	Binds to the 50S ribosomal subunit and inhibits ribosomal peptide bond formation.
Carbenicillin	50	H ₂ O or 50% EtOH	-20° C	50	1.0 µl/ml	Inhibits bacterial cell wall synthesis.
Chloramphenicol	35	EtOH or MetOH	-20° C	35	1.0 µl/ml	Inhibits protein synthesis.
D-cycloserine*	10	0.1M NaPO ₄ pH8.0	+4°C	10	1.0 µl/ml	Competitively inhibits alanine racemase and D-alanine ligase.
Erythromycin	20	EtOH	-20° C	20	1.0 µl/ml	Binds to the 50s subunit of the bacterial 70s rRNA, preventing protein synthesis.
Gentamycin	10	H ₂ O	-20° C	10	1.0 µl/ml	Inhibits protein synthesis by binding to L6 protein of the 50S ribosomal subunit.
Hygromycin B	10	H ₂ O	-20° C	400	40 µl/ml	Inhibits protein synthesis.
Kanamycin	50	H ₂ O	+4°C- - 20° C	50	1.0 µl/ml	Interacts with at least three ribosomal proteins, inhibiting protein synthesis and increasing translation errors.
Kasugamycin	10	H ₂ O	-20° C	10	1.0 µl/ml	Inhibits protein synthesis at the step of translation initiation by competing with initiator transfer RNA.
Nalidixic acid	30	H ₂ O: pH to 11 w/ NaOH	-20° C	30	1.0 µl/ml	Binds to the A subunit of DNA gyrase (topoisomerase) and prevents supercoiling of DNA, thereby inhibiting DNA synthesis.
Neomycin	10	H ₂ O	-20° C	800	80 µl/ml	Binds to ribosomal components and inhibits protein synthesis.
Rifampicin**	50	MetOH	-20° C	100	2.0 µl/ml	Inhibits prokaryotic RNA polymerase.
Spectinomycin	100	H ₂ O	-20° C	100	1.0 µl/ml	Inhibits translocation of the peptidyl tRNA from the A site to the P site.
Streptomycin	100	H ₂ O	-20° C	100	1.0 µl/ml	Inhibits protein synthesis; binds to 30S ribosomal subunit.

Tetracycline***	15	EtOH, 70%EtOH	-20°C	15	1.0 µl/ml	Inhibits growth by preventing codon-anticodon interactions during translation.
Triclosan	10	EtOH	-20°C	10	1.0 µl/ml	Inhibits fatty acid synthesis.
Trimethoprim	10	10% EtOH or DMSO*****	-20° C	10	1.0 µl/ml	Inhibits dihydrofolate reductase so bacteria cannot take up folate.

* - Unstable solution - prepare just before use.

** - Light sensitive – wrap container in foil.

*** - Light sensitive, Mg²⁺ - inhibitor - do not use with minimal media.

**** - Filter sterilize solutions into 1 ml aliquots before storage.

***** - DMSO (di-methyl sulfoxide) will dissolve cellulose acetate membranes commonly used for filter-sterilization, so use nylon membranes.

Most antibiotics should be ordered as the salt or HCl preparation, i.e., “ampicillin-*sodium salt*” or “tetracycline-*HCl*”. These dissolve much easier than the other preparations.

Method

1. Prepare and autoclave/sterilize stock media. Be sure that the flask contains a stir-bar.
2. The solution must cool before adding antibiotics as the heat may inactivate them. Let the flask equilibrate in the water bath set at 55-60° C for a minimum of 30 min. At this point, agar solutions should be warm enough that it won't soon solidify, but cool enough that it won't inactivate the antibiotics. Liquid broths will not solidify when they cool.
3. Most of the stock solutions above are prepared at a 1000X concentration are used to select for common Gram-negative or Gram-positive organisms that might carry resistance plasmids or other resistance genes (as opposed to susceptible strains that lack these genes). In most cases you will add 1 ml of stock antibiotic per liter of solution. If you prepared 500 ml of media, add 500 µL of antibiotic stock solution, etc. Use a sterile pipette to add the solution. Adjust the added volume of stock solution accordingly to change the final antibiotic concentration, if desired.
4. Using a stir-plate, swirl the media and antibiotic to mix thoroughly. Try not to introduce air bubbles.
5. For agar preparations, pour the plates, carefully stack up to ten high and let solidify. Keep at room temperature overnight, then refrigerate in clean plastic sleeves until use. If the plates will be stored in ambient light for some period prior to use, cover the stack of plates with tinfoil, as light inactivates many antibiotics.