

ANTIBIOTICS

A. Antibiotic Concentrations.

Antibiotic	Abbreviation	Final concentration ¹		Stock solution ²
		Rich media	Minimal media	
Na Ampicillin ³	Amp or Ap	30 $\mu\text{g/ml}$	15 $\mu\text{g/ml}$	9 mg/ml
Chloramphenicol	Cam or Cm	20 $\mu\text{g/ml}$	5 $\mu\text{g/ml}$	6 mg/ml
Gentamycin SO ₄	Gen or Gm	10 $\mu\text{g/ml}$	----	3 mg/ml
Kanamycin SO ₄ ⁴	Kan or Km	50 $\mu\text{g/ml}$	125 $\mu\text{g/ml}$	15 mg/ml
Tetracycline HCl ⁵	Tet or Tc	20 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	6 mg/ml
Timentin	---	80 $\mu\text{g/ml}$	---	24 mg/ml
Trimethoprim	Tm	200 $\mu\text{g/ml}$	---	15 mg/ml
Spectinomycin	Spc or Sp	100 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	50 mg/ml
Streptomycin SO ₄ ⁶	Str or Sm	---	2 mg/ml	---

¹ The solid form of the antibiotics can be added directly to sterilized media that has been cooled to approximately 55°C. If kept at 4°C tetracycline, chloramphenicol, and streptomycin plates are usually good for several months, but kanamycin plates and ampicillin plates may only last for several weeks. A sensitive and resistant control should always be included each time antibiotic plates are used.

² For liquid media or for a few plates, a stock solution of the antibiotics can be prepared and stored at -20°C. All the antibiotic stock solutions can be prepared in sterile dH₂O except chloramphenicol which can be dissolved in dimethylformamide and timentin which can be dissolved in 70% ethanol. The stock solution is 300x the concentration of antibiotic required in rich medium. An average petrie dish contains about 30 ml medium, so spread 0.1 ml on rich plates.

³ High level expression of β -lactamase can destroy ampicillin in the medium surrounding the Amp^R colonies, allowing Amp^S satellite colonies to grow. Satellite colonies can be decreased by using 2x ampicillin when selecting for plasmids. Alternatively, Timentin prevents growth of satellite colonies.

⁴ To select for a Kan^R gene on a multicopy plasmid use LB + 10 mM Tris HCl pH 7.4 + 250 $\mu\text{g/ml}$ Kanamycin SO₄ or Neomycin SO₄. High levels of Kan^R are not expressed if the pH of the medium is less than 7.2.

⁵ Certain strains are only resistant to half this concentration of tetracycline.

⁶ For streptomycin plates add 1% Nutrient broth to the minimal media (i.e., 1 ml NB/100 ml medium).

B. Antibiotic sensitivity and resistance.

Ampicillin is a penicillin derivative that inhibits crosslinking of peptidoglycan chains in the cell wall of eubacteria. Cells growing in the presence of ampicillin synthesize weak cell walls, causing them to burst due to the high internal osmotic pressure. Amp^R encoded by Mu derivatives and pBR plasmids is due to a periplasmic β -lactamase that breaks the β -lactam ring of penicillin derivatives.

Chloramphenicol inhibits protein synthesis by binding to the 50s ribosomal subunit and blocking the peptidyltransferase reaction. Cam^R encoded by pBR328 is due to a cytoplasmic chloramphenicol acyltransferase which inactivates chloramphenicol by covalently acetylating it.

Gentamycin is an aminoglycoside antibiotic that inhibits protein synthesis by binding to the ribosome and preventing translocation. Although it is readily transported into many bacteria, gentamycin does not efficiently cross eukaryotic cytoplasmic membrane. (This contrast is probably due to differences in membrane potential maintained across the bacterial cytoplasmic membrane vs the eukaryotic cytoplasmic membrane). Gen^R is usually due to a plasmid encoded enzyme that inactivates aminoglycosides by covalent modification.

Kanamycin inhibits protein synthesis by binding to the 30s ribosomal subunit and preventing translocation. Kan^R is usually due to a cytoplasmic aminoglycoside phosphotransferase that inactivates kanamycin by covalently phosphorylating it. Kan^R requires phenotypic expression. Neomycin is a structural analog of Kanamycin that functions by the same mechanism and is inactivated by the same mechanism.

Tetracycline inhibits protein synthesis. Tetracycline reversibly binds to the small subunit of ribosomes and interfere with binding of aminoacyl-tRNA to the Acceptor site. Tetracycline is bacteriostatic in bacteria. Tetracyclines can also inhibit protein synthesis in eukaryotes, but are less likely to reach inhibitory concentrations because eukaryotes lack a tetracycline uptake mechanism. Tet^R encoded by Tn10 and pBR plasmids is due to a membrane protein that actively exports tetracycline out of the cell. When Tn10 is present in multiple copies, cells are less resistant to Tet than when only one copy of Tn10 is present.

Timentin is a mixture of the penicillin derivative Ticarcillin and the β -lactamase inhibitor, clavulanic acid. Timentin is useful for selecting for penicillin resistance in cultures of gram negative bacteria that secrete large amounts of β -lactamase – resistant cells are protected by β -lactamase retained within the periplasm, while exogenous β -lactamase is inactivated by the clavulinic acid, allowing the Ticarcillin to inhibit growth of any sensitive cells in the population. One use of Timentin is to inhibit growth of penicillin-sensitive satellite colonies.

Trimethoprim is an inhibitor of bacterial dihydrofolic acid reductase. Resistance to trimethoprim can arise by chromosomal mutations that cause overproduction of dihydrofolate or plasmid encoded trimethoprim-resistant dihydrofolate reductases.

Spectinomycin inhibits protein synthesis by binding to the Sr protein of the 30s ribosomal subunit and blocking translation. Spectinomycin resistance can arise via a single base

substitution in the chromosomal gene encoding this ribosomal protein (*rpsE*). Plasmid encoded resistance to both spectinomycin and streptomycin can arise via modification of the antibiotics – for example, the *aad* gene transfers an adenyl group from ATP to these antibiotics which inactivates the antibiotic.

Streptomycin inhibits protein synthesis by binding to the S12 protein of the 30s ribosomal subunit and blocking translation. A high level of Strr can result from chromosomal mutations in the gene for the S12 protein (*rpsL*) which prevent streptomycin from binding to the ribosome. Since only mutant ribosomes are Str^R, resistance to streptomycin is recessive to streptomycin sensitivity. Str^R requires phenotypic expression.

REFERENCES

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