Common Stocks, Antibiotics

Stock, conc	Preparation & Storage (Bennett Lab)	Volumes, Tubes	Location
10× T4 ligase buffer	Thawed, precipitates resuspended with vortexing/slight warming.	50 µL aliquots	Rack 1.5, -20°
10 mM dNTPs		20 µL aliquots	Rack 1.5, -20°
Antibiotics			
chl$^{25\ (mg/mL)}$	Ethanol, -20° liquid; stable for 1 year.	15 mL	Fr 1 door
amp^{100}	40% ethanol, -20° liquid; stable enough for 6 mo [8]. Definitely in water, -20° solid for 6 mo [10,11].	15 mL	Fr 1 door
kan^{50}	Water, 0° liquid; stable for 1 year.	15 mL	Fr 0 door
spt^{50}	Water, 0° liquid; stable for 1 year.	15 mL	Fr 0 door
gen^{10}	Water, -20° solid; stable for 1 year.	~1 mL	Rack 1.5
str^{100}	Water, -20° solid; stable for 1 year.	~1 mL	Rack 1.5, -20°
tet^{10}	Ethanol, -20° liquid; stable in dark for 1 year?	~1 mL	Rack 1.5, -20°
zeo^{100}	?, -20° solid; stable in dark for 1 year.	~1 mL	Rack 1.5, -20°

Antibiotics

Antibiotic	Class, Target	Abbr*	Stock Concentration		Solvents, Temp	Notes	Rich Medium, common *E. coli*				Common resistance genes
			m/V	Relative (*E. coli*)			1× *c*, Multi-Copy; Range		1× *c*, Single-Copy	Stability	
Chloramphenicol	misc, ribosome	**chl**	25 mg/mL	1000×	Ethanol, -20°(*l*)	ChlR gene is toxic to *E. coli* in minimal media	25 or 34 mg/L[3]	25–340 mg/L [5]	10 mg/L [1] or 5 mg/L [6]	*All*: 1 yr [8–10]	Chloramphenicol acetyltransferase Type A1, *cat* .
Ampicillin·Na Carbenicillin·2Na	penicillin, peptidoglycan	**amp** **car**	100 mg/mL	1000×	40% ethanol, -20°(*l*) or water, -20°(*s*)	40% EtOH keeps it liquid at -20°C. Substitutes carbenicillin.	100 mg/L[3]	100–200 mg/L	50 mg/L	*Stock*: 6 mo with <¼ loss in water [10, 11] . *Agar*: selective for 4 mo [8], 44% loss every 4 mo [9].	-lactamase (*bla*).
Kanamycin A·SO_4	aminoglycoside, ribosome	**kan**	50 mg/mL	1000×	Water, 0°(*l*); -20°(*s*)	Insoluble in 40% ethanol. Precipitates in 35% DMSO.	50 mg/L[3]	50–500 mg/L [5]	20 mg/L [1]	*All*: 1 yr [8–10]	Aminoglycoside 3-phosphotransferase Type I or II, *aphA1* / *aph(3')-I* / *nptI*, *aph(3')-II* / *nptII*. Type IIIa works in Gram(+).
Spectinomycin·2HCl·5H_2O	aminoglycoside, ribosome	**spt**	50 mg/mL	1000×	Water, 0°(*l*); -20°(*s*)	Precipitates in 35% DMSO. Using 120 mg/L prevents spontaneous genomic resistance evolving [13].	50 mg/L[3] or 120 mg/L[13]	50–500 mg/L [5]	20 mg/L [1]	*All*: 1 yr [8–10]	Aminoglycoside 3-adenylyltransferase, *aadA*.
Streptomycin·SO_4	aminoglycoside, ribosome	**str**	100 mg/mL	1000×	Water, 0°(*l*); -20°(*s*)	Genomic resistance via *rpsL* mutation is common in lab strains.	100 mg/L			*All*: 1 yr [8–10]	Plasmids: Aminoglycoside 3-adenylyltransferase, *aadA*. Genomic resistance usually *rpsL* mutant.
Trimethoprim	diaminopyrimidine, FolA (DHFR)	**tmp**	15 mg/mL	1000×	DMSO or 1% acetic acid	Insoluble in water or ethanol. Exogenous thymine can lead to evolution of resistance.	15 mg/L?		10 mg/L [2]	?	Dihydrofolate reductase type II, *dfrB2* .
Apramycin·SO_4	aminoglycoside, ribosome	**apr**	?	1000×	Water	.	?		?	*All* : 1 yr [8–10]	Aminoglycoside N^3-acetyltransferase, *aac(3)-IV* / *aacC4* .

Gentamicin C $\cdot SO_4$	aminoglycoside, ribosome	**gen**	10 mg /mL?	1000×	Water	.	10 mg /L [8]		10 mg/L [1]	*All* : 1 yr [8–10]	Aminoglycoside-N^3-acetyltransferase, *aac(3)-I / aacC1* .
Erythromycin	macrolide, ribosome	**ery**	50 mg /mL	*100*×	Water, ethanol, DMSO	.	500 mg/L		200 mg/L [12]	?	23S rRNA(adenine^{2085}) N^6-methyltransferase, *ermC* .
Tetracycline·HCl	tetracycline, ribosome	**tet**	10 mg /mL	1000×	Ethanol	Photolabile.	10 mg /L [3]	10–15 mg/L	7.5–15 mg /L [1,12]	*Stock*: 4° 1 wk, -20° 2 mo [10]	metal:tetracycline/H$^+$ antiporter, class C, *tetA*(C).
Doxycycline	tetracycline, ribosome	**dox**			Ethanol	Photolabile?					?
Zeocin (Phleomycin D1·Cu^{2+})	glycopeptide, DNA	**zeo**	25 /100 mg /mL	1000×	Water	Use 25 mg/L with LB-Lennox (5 g/L NaCl), pH 7.5. Or use 100 mg/L for high-salt LB. Light-sensitive. Limit freeze-thaws.	25 mg /L [7, low-salt] 100 mg/L	25–50 mg /L [7, low-salt]		*Agar*: 1 mo (Invivogen)	bleomycin/phleomycin binding protein, *ble*.
Hygromycin B	aminoglycoside, ribosome	**hyg**	200 mg /mL	1000×	Water	.	50-200 mg/L [3]			?	Hygromycin B phosphotransferase, *hph* .

Note: aminoglycoside solutions are generally found to be stable for more than a year in 4° water or agar [8–10].

* Follow American Society of Microbiology AAC abbreviation guidelines

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13. Barrick Lab wiki: antibiotics

Lab Improvement Weekly: Investigative Report on Ampicillin. By Shyam Bhakta.

This week's investigative report examines everyone's favorite yet hated bacteriolytic antibiotic: ampicillin.

A 1990 paper[1] discusses how the secreted -lactamase in a saturated preculture of an Amp strain can quickly consume all the ampicillin in a subculture. A stationary culture of ampicillin resistant cells can have such a concentration of -lactamase that even a 1/200 – 1/1000 dilution will still contain enough -lactamase to consume all the fresh ampicillin before all the non-resistant cells from the stationary phase culture have been killed. Even without any initial -lactamase, enough -lactamase is secreted quickly that toxic plasmids can quickly be lost midway through growth without facing selection. The authors recommend not allowing cultures to reach stationary phase if you need a high proportion of cells to contain your plasmid. And before subculturing, cells are best washed for Amp selection.

Ampicillin's bactericidal activity has a pH optimum is 5.5–6 and is tenfold less at pH 8 [3].

A paper on stability of antibiotics in solid medium over time[2] found that 4 wk-old amp plates stored cold have a 30–50% loss in [amp] (causing a 10% change in zone of inhibition size), BUT no change (especially at our high concentration) that would dip below typical Amp^S strain's MIC. Two-month-old plates would seem to be the limit, as after the resulting fourfold reduction in [amp] would approach the MIC. The rest of our common four antibiotics have no significant changes in agar over 2 months.

Activity reduction of a 100 mg/mL (1000×) aqueous, unbuffered solution of ampicillin[3] :

4°   20° -20°

1 d: 15%, 28%

2 d: 33%, 45%

7 d: 65%, 81%

6 mo: <10%

A number of studies have indicated that the stability of ampicillin in solution appears to be a function of pH, temperature, and even the identity of the buffer. Although ampicillin in any form is more readily soluble in base, it rapidly loses activity when stored above pH 7.0. Optimal conditions for storage were suggested as 2–8°C, pH 3.8–5, retaining more than 90% activity for a week. Another review noted that the buffer used can also affect stability: at pH 7, Tris is "highly deleterious to the stability… but not so at pH 5;" citrate is fine at pH 7 but not at pH 5; acetate buffer seems best at pH 6.5 .

Carbenicillin is anecdotally more chemically stable in media, especially acidic and hot, and more resistant to -lactamase, but I can only find published evidence that supports the former ; I'm suspicious/doubt the latter claim. Maybe more carb survives 50° agar, but more amp (I've read 200 mg/L) can be used if it matters. While media stability ought to give carbenicillin an edge in selection against amp-sensitive mutants over long culture periods, especially in acidifying media (glucose carbon source), peptide broths like LB alkalify over culture time, and many-day culturing or a requirement of maintenance of 1× concentration in sterile media is rare. I've found 1× amp in LB30/TB liquid is selective for cloning for at least 2 wk in the fridge, longer for regular LB. The remaining amp only needs to exceed the MIC by a fewfold. 100–200 mg /L is 8–16-fold the MIC.

A new paper deletes a small part of the AmpR RBS so it makes less -lactamase. Lowers how much amp is needed and it reduces -lactamase accumulation to the level that subcultures of AmpR strains from amp to fresh amp medium preserves selection without quickly destroying all the amp .

[1] https://www.ncbi.nlm.nih.gov/pubmed/2199796?dopt=Abstract

[2] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC376956/pdf /applmicro00107-0177.pdf

[3] https://www.applichem.com/en/shop/product-detail/as/ampicillin-natriumsalz-ibiochemicai/

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