

Media amendments

Antibiotic concentrations and stock solutions

This table lists “standard” concentrations of antibiotics commonly used in microbiology laboratories. These concentrations apply to *E. coli*. Antibiotic selection in other organisms may use different concentrations, so check primary literature for more info.

Reagent	Abb	Stock Conc.	Working Conc.	Dilution	Solvent	MSDS	Notes
Ampicillin	Amp	100 mg/mL	100 µg/mL	1,000×	ddH ₂ O	link	Ampicillin degrades quickly in both plates and stock solutions. Culture plates with Amp can be stored at 4°C for about 2 weeks. Stock solutions can be stored at 4°C for 2 weeks but can last as long as 4-6 months when stored at -20°C. In general, use carbenicillin for ampicillin selection, because it is more chemically stable.
Carbenicillin	Crb	100 mg/mL	100 µg/mL	1,000×	50% EtOH	link	Carbenicillin can be used in place of Ampicillin at the same working concentration. Carbenicillin is more stable than Ampicillin, and is generally preferable.
Chloramphenicol	Cam	20 mg/mL	20 µg/mL	1,000×	EtOH	link	
Cycloheximide	Chx	100 mg/mL	100 µg/mL	1,000×	100% DMSO	link	Anti-fungal, toxic to humans, use PPE!
D-Cycloserine	Dcs	4 mg/mL	4 µg/mL	1,000×	ddH ₂ O	link	Degrades noticeably over the course of 1 week at 4°C. D-cycloserine is best used within two days of pouring. A single small-scale experiment found 6-10x as many <i>E. coli</i> colonies on 8-day old MG-Dcs plates that had spent 3 of those days at -20°C and 5 at 4°C, compared to what was found on 1 day old MG-Dcs.
Erythromycin	Ery	250 mg/mL	250 µg/mL	1,000x	EtOH	link	May crystallize when added to liquid culture. Still works. Selection strength may vary in non-model bacteria
Gentamicin	Gen	20 mg/mL	20 µg/mL	1,000×	ddH ₂ O	link	For non-canonical experiments: Use 20 µg/mL in culture from 20mg/mL 1000× stock
G418	G418	200 mg/mL	200 µg/mL	1000x	ddH ₂ O	link	Geneticin generic. Powder store RT. Stock solution at -20°C
Kanamycin	Kan	50 mg/mL	50 µg/mL	1,000×	ddH ₂ O	link	Plates start to lose sensitivity after 2 weeks; recommended Kanamycin plates only last a few weeks at 4°C; best to make fresh Kan plates every 2 - 4 weeks.
Nalidixic Acid	Nal	30 mg/mL	30 µg/mL	1,000×	1M NaOH	link	
Novobiocin	Nov	200 mg/mL	200 µg/mL	1,000×	ddH ₂ O	link	
Rifampicin	Rif	50 mg/mL	100 µg/mL	500×	MeOH + drops of 10M NaOH	link	Toxic to humans, use PPE! Rifampicin is TOXIC to humans, light-sensitive, and degrades in solution rather quickly. It needs to be stored wrapped in foil and replaced every 2 months.
Spectinomycin	Spec	60 mg/mL	60 µg/mL	1,000×	ddH ₂ O	link	Common to see background growth of cells on selective plates. Use at a 120 µg/mL working concentration when cloning!! (double what you would normally add to your media)
Streptomycin	Str	100 mg/mL	100 µg/mL	1,000×	ddH ₂ O	link	
Tetracycline	Tet	10 mg/mL	10 µg/mL	1,000×	70% EtOH	link	Light sensitive, stability < 2 weeks
Trimethoprim	TMP	10 mg/mL	variable?	variable	DMSO	link	Both 10 and 20 µg/ml listed for use. Use as counter selectable marker for mutations in <i>thyA</i> requires presence of thymine/thymidine in media
Zeocin	Zeo	25 mg/mL	25 µg/mL	1,000×	ddH ₂ O	link	Prodcut literature lists active range of 25 - 50 µg/ml for <i>E. coli</i> and 50 - 300 in yeast. In our hands we note:

10 µg/mL is specifically used for growth of amberless strain A37. For yeast, 100µg / ml is sufficient.

Other common media amendments

Reagent	Abb	Stock Conc.	Working Conc.	Dilution	Solvent	MSDS	Notes
5-fluorouracil	5FU	13 mg/mL	13 µg/mL	1,000×	DMSO	link	100mM stock concentration 100µM working concentration
Anhydrotetracycline	ATC	0.1 mg/mL	100 ng/mL	1,000×	DMSO	link	Can also make a 1mg/mL stock and dilute 1:10 for a final concentration of 0.1mg/mL
3'-Azido-2',3'-dideoxythymidine	AZT	10 mg/mL	200 µg/mL	50×	ddH ₂ O	link	Can substitute pharma grade Zidovudine. Soluble up to 20 mg/mL in ddH ₂ O. Very unstable in solution. Store at -20°C and activity will still decrease over weeks of storage.
Bromo-chloro-indolyl-galactopyranoside	X-gal	40 mg/mL	80 µg/mL	500×	Dimethylformamide (DMF)	link	X-gal is a histochemical substrate for β-galactosidase, which cleaves X-Gal to yield an insoluble blue precipitate.
Diaminopimelic acid	DAP	60 mM	0.3 mM	200×	ddH ₂ O	link	Use 5 µl stock per 1 ml of media. Dissolve ~30min shaking at 37C
Isopropyl β-D-1-thiogalactopyranoside	IPTG	0.1 M	0.1 mM	1,000×	ddH ₂ O	link	Light sensitive, MW: 238.31 g/mol
Triphenyl Tetrazolium Chloride	TTC	5% (50 mg/mL)	0.05% (50 µg/mL)	1,000×	ddH ₂ O	link	Used in growth/sugar-fermentation indicator agars. Filter sterilize. Light sensitive. Store stock solution at 4°C wrapped in foil. Use at ten-fold higher concentration in motility agar (0.5%).

Working concentrations are for liquid or solid media.

Preparation

* Generally, prepare 30-50 mL of solution in a 50 mL conical tube. Then, filter sterilize solutions by pushing them through a 50 mL syringe fitted with a 0.22 µm filter made of a material appropriate for the solvent. Compounds dissolved in Ethanol or DMSO do not require filter sterilization. Aliquot into 1.5 mL portions in 1.7 mL tubes using the repeat pipettor. Label with a permanent marker (one that won't wash off with ethanol). Store at -20°C in the dark.


Non-canonical amino acid stock solutions

Amino Acid (Abbreviation)	CAS Number	Solvent	MW (g/mol)	[Stock]	[Stock] (mg/mL)	[Culture]	Notes
Y	60-18-4	Water	181.19	10mM	1.812	250µM	Requires heating to 70°C to dissolve Stock precipitates out of solution at or below room temperature
IodoY	70-78-0	Water	307.09	10mM	3.071	250µM	Dissolves at room temperature with ~1 hour of shaking
AminoY (FAA)	300-34-5		196.203				
AminoY (HS)	23279-22-3	Water	287.14	10mM		250µM	Dissolves readily without shaking or heat
BromoY	38739-13-8	Water	260.0846	10mM		250µM	
ChloroY	7423-93-0	Water	215.63	10mM		250µM	
NitroY	621-44-3	Water	226.2	10mM	2.262	250µM	Will dissolve with heating to 50°C, no shaking
ONBY (FAA)	184591-51-3		316.31				Light sensitive
ONBY (S)	207727-86-4	50% DMSO 50% Water (exothermic)	352.77	50 mM		250µM	Light sensitive Requires adding OH ⁻ to dissolve
AzF	33173-53-4	10% DMSO 90% Water	206.204	10mM	2.062	250µM	Light sensitive Requires heating and shaking to dissolve
CNF	167479-78-9	Water	190.2	10mM	1.902	250µM	Dissolves at room temperature with ~15 minutes of shaking
L-DOPA	59-92-7	Water	197.1879	10mM	1.972	250µM	Oxidizes quickly and turns black in solution
5HTP	4350-09-8	Water	220.2246	10mM	2.202	250µM	


FAA: free amino acid

S: salt

HS: hydrated salt

I	Attachment	History	Action	Size	Date	Who
	Making_stock_antibiotics_and_other_reagents.docx	r1	manage	89.7 K	2019-01-11 - 21:56	KateElston

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