ANTIBIOTIC SELECTION, PREPARATION AND STORAGE

In general, antibiotics require storage in a refrigerator or freezer. Aminoglycosides (e.g., kanamycin) are hygroscopic and should be stored in a desiccator. All antibiotics should be protected from direct sunlight. Rifampicin and Amphotericin B are very sensitive to light and should be stored in the dark.

The relationship between the weight (mg) of antibiotic, the activity of the powder (µg/mg or units per mg), the volume of solution to prepare (mL), and the concentration (µg/mL) of antibiotic desired in the solution is:

$$\text{Weight} = \frac{\text{Volume X Concentration}}{\text{Activity}}$$

Most antibiotic solutions will remain stable stored at -0°C for up to 3 months (unless otherwise noted on the following table). However, Rifampicin and Tetracyline should be freshly prepared for each use. Most antibiotics are heat liable and should be filter sterilized generally using 0.2 µm hydrophilic membranes.

Different plant species exhibit different sensitivities t o antibiotics. For example, one antibiotic may have minimal toxicity to certain plant species while being extremely toxic to other species. For this reason, recommended concentrations for antibiotic use are not included in the following table. Typically, antibiotics are used at concentrations at or above that toxic to the target microbes.

CARBENICILLIN PREPARATION

(Product No. C346, C540)
Carbenicillin is a white to off-white, hygroscopic powder that is soluble in water. Carbenicillin is most effective against gram-negative bacteria but may also have some effect against gram-positive bacteria. Aqueous solutions of Carbenicillin are reported to be stable for 40 hours at 35°C at pH 5.5. Solutions can be stored for 24 months -20° C (i.e., non-frostfree freezer). Carbenicillin has a relatively low toxicity to a wide range of plant species; concentrations of up to 1000 mg/L have been reportedly used in plant tissue culture.

CEFOTAXIME PREPARATION

(Product No. C380, C537, and C1880)
Cefotaxime is a white to off-white powder, which is freely soluble in water. Variations in color of the freshly prepared solutions do not necessarily indicate changes in potency. Store this product in an airtight container protected from light. Cefotaxime is amongst the most stable of all β-lactam ring containing antibiotics, and generally resistant to most β-lactamases. Aqueous solutions of Cefotaxime are most stable at a pH range of 4.3-6.2. Solutions can be stored for 48 months at -20° C (i.e., non-frostfree freezer). Cefotaxime is most effective against gram-negative bacteria.

GENETICIN® [Antibiotic G418]

(Product No. G810)
Although it is related to Gentamicin, Geneticin is not normally used as a standard antibiotic. Its most common application is in molecular biology as a selection agent. Geneticin, also known as antibiotic G418 sulfate, is toxic to bacteria, yeast, protozoa, helminthes, and mammalian cells. Resistance is conferred by one of two dominant genes of bacterial origin, which can also be expressed in eukaryotic cells.

Geneticin is water-soluble and is stable when stored as a dry powder at 2-6°C for 3 years. Aqueous solutions are stable for 2 years at 2-6°C. The amount of Geneticin required for selection will vary with each cell type and growth cycle. Although cells that are multiplying will be affected sooner than those that are not; cells that are in log phase will still require 3 to 7 days for selection.

Concentrations for use with plant cells have been reported to be as low as 12.5-50 µg/mL. This is significantly lower than typical concentrations of 200-400 µg/mL used with mammalian cells.

Geneticin® is a registered trademark of Invitrogen, Inc.

HYGROMYCIN B

(Product No. H370, H385, & H397)
Hygromycin B is an aminoglycoside antibiotic, which is effective against prokaryotic and eukaryotic microorganisms and cells. Similar to Geneticin®, its most common application is in molecular biology as a selection agent. Cells transformed with the hph gene are resistant to Hygromycin B.

Hygromycin B is provided as a 100 mg/mL aqueous solution (H370, H385) with an average potency of 1,000 units/mg and as a powder (H397). The recommended concentration range for use as a selection agent is 10 - 400 µg/mL. Stock solutions can be stored for at least 1 year at 2-6° C; solutions should NOT be frozen as this can reduce their potency.

Typical selection concentrations:
- Prokaryotes – 100 µg/mL
- Lower eukaryotes – 200 µg/mL
- Higher eukaryotes – 150-400 µg/mL

Refer to the following antibiotic/ antimycotic/ selection agent guide and pertinent scientific references for more specific application information on these and other antibiotics.

_Phyto_Technology Laboratories®
P.O. Box 12205; Shawnee Mission, KS 66282-2205
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Web Site: www.phytotechlab.com

*Phyto*Technology Laboratories® Technical Information

ANTIBIOTIC/ ANTIMYCOTIC/ SELECTION AGENT GUIDE

Product Name	Prod. No.	Mol. Wt.	Gram (+) Bacteria	Gram (-) Bacteria	Mycobacteria	Fungi	Yeast	Mycoplasma	Selection Agent	Microbe Toxicity (µg/mL)	Toxicity to Plant Tissues[1] (µg/mL)	Solubility	Store Soln[2]
Amphotericin B	A119	924.1				++	++			2.5	>5	DMSO	R
Ampicillin	A116	371.4	++	++					++	50	100	Water	F
Amoxicillin	A122	419.5	+	++						Varies	-	Water	R
Bacitracin Zinc	B132	1421.6	++							50	150	Water	F
Carbenicillin	C346	422.4	+	++						500	>1000	Water	F
Carbenicillin Solution (100 mg/mL)	C540												
Carbendazim	C1888	191.19				++	++					HCL	
Cycloheximide	C1989	281.36				++	++					DMSO	
Cefotaxime	C380	477.4	+	++						90	>100	Water	F
Cefotaxime Solution (100 mg/mL)	C537												
Cefotaxime Solution (250 mg/mL)	C1880												
Cephalexin	C1970	365.4	++	+								Water	F
Chloramphenicol	C252	323.1	++	++	+			+	++	128	1-64	EtOH	R
Chloramphenicol Solution (10 mg/mL)	C2010												
Erythromycin	E344	733.9	++	++						0.5-30	150	Water	R
G418	G810	692.7							++		50	Water	R
Gentamicin	G570	575.7	+	++				++		50	80	Water	R
Gentamicin Solution (50 mg/mL)	G3350												
Gentamicin Solution (100 mg/mL)	G3410												
Hygromycin B	H397	527.5							++	NA	20-400	Water	R
Hygromycin B Solution (PBS)	H370												
Hygromycin B Solution (Water)	H385												
Kanamycin	K378	582.6	++	++				++	++	100	2	Water	R
Kanamycin Solution (50 mg/mL)	K586												
Kanamycin Solution (100 mg/mL)	K4751												
Neomycin	N584	908.9	++	++					++	50	900	Water	R
Neomycin Solution (50 mg/mL)	N5967												
Nystatin	N581	926.1				++	++			50	40	DMSO	F
Paromomycin	P710	713.7	++						++		50	Water	R
Penicillin G	P777	356.4	++							Varies	100	Water	F
Pentachloronitrobenzene (PCNB)	P6737	295.33				++			++			DMSO	RT
Polymyxin B	P6809	1385.6		++								Water	R
Ribavirin	R795	244.2											
Rifampicin	R501	822.9	++	++	++					15	100	Water	F
Spectinomycin	S742	405.3	+	++					++	20	500	Water	F
Streptomycin Sulfate	S739	1457	++	++						100	16	Water	F
Streptomycin Solution (250 mg/mL)	S7739												
Tetracyline	T859	480.9	++	++						10	50	Water	F[3]
Tetracycline HCl Solution (10 mg/mL)	T7859												
Timentin	T869	NA		++					++		200	Water	F
Timentin Solution (50 mg/mL)	T7869												
Timentin Solution (100 mg/mL)	T767												
Tobramycin	T834	1425.5		+								Water	
Tyrothricin	T8110	1228.4	+						+			EtOH	R
Vancomycin	V870	1485	++							5	80	Water	R
Vancomycin Solution (100 mg/mL)	V8370												

++ = Effective against most microorganisms, + = Effective against certain microorganisms

[1]The concentrations for plant toxicity noted in the table may be higher or lower for different plant species due to the great differences between species in toxic sensitivity to antibiotics. A concentration showing no toxicity to one plant species may exceed the toxic concentration in a different species.
[2]Solution storage: F = Freezer; R = Refrigerator; RT = Room Temperature
[3]Aqueous Solutions of Tetracycline (pH>3) epimerize (even if frozen) yielding a hazy appearance. Solutions should only be stored (frozen) for short periods, e.g., one week. Preparation of fresh solutions is recommended.

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