



PhytoTechnology Laboratories™

Dedicated to a Better Way of Life Through Plants™

ANTIBIOTIC 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. Storage of many powdered antibiotics at -20° C is not recommended and increases the risk of water condensation. 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 ($\mu\text{g}/\text{mg}$ or units per mg), the volume of solution to prepare (mL), and the concentration ($\mu\text{g}/\text{mL}$) of antibiotic desired in the solution is:

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

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 Tetracycline should be freshly prepared for each use.

Most antibiotics are heat liable and should be filter sterilized using 0.2 μm (or smaller) low-binding membranes (e.g., cellulose acetate, teflon).

CARBENICILLIN PREPARATION (Product No. C346)

Carbenicillin is a white to off-white, hygroscopic powder that is soluble in either water or alcohol. 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 up to 24 hours at room temperature and for up to 72 hours when stored at 2-6° C. Solutions can be stored for 12 months at a stable -20° C (i.e., non-frostfree freezer). Carbenicillin has a relatively low toxicity to a wide range of plant species; concentrations of up to 500 mg/L have been reportedly used in plant tissue culture.

CEFOTAXIME PREPARATION (Product No. C380)

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. Aqueous solutions of Cefotaxime at a pH of 4.5-6.2 are stable for 14-21 days when stored at 2-6° C. Solutions can be stored for 12 months at a stable -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 can be stored at room temperature for as long as 1 year. Aqueous solutions should be stored frozen. 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 $\mu\text{g}/\text{mL}$. This is significantly lower than typical concentrations of 200-400 $\mu\text{g}/\text{mL}$ used with mammalian cells.

Geneticin® is a registered trademark of Invitrogen, Inc.

HYGROMYCIN B (Product No. H370)

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 with an average potency of 1,000 units/mg. The recommended concentration range for use as a selection agent is 10 - 400 $\mu\text{g}/\text{mL}$. 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 $\mu\text{g}/\text{mL}$
- Lower eukaryotes – 200 $\mu\text{g}/\text{mL}$
- Higher eukaryotes – 150-400 $\mu\text{g}/\text{mL}$

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

PhytoTechnology Laboratories Technical Information

ANTIBIOTIC/ ANTIMYCOTIC/ SELECTION AGENT GUIDE

Different plant species exhibit different sensitivities to 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.

Product	Prod. No.	Mol. Wt.	Gram (+) Bacteria	Gram (-) Bacteria	Mycobacteria	Fungi	Yeast	Mycoplasma	Selection Agent	Microbe Toxicity (ug/mL)	Toxicity to Plant Tissues ¹ (ug/mL)	Solubility	Store Soln ²
Amphotericin B	A119	924.1				++	++			2.5	>5	DMSO	R
Ampicillin	A116	371.4	++	++					++	50	100	Water	F
Bialaphos	B131	345.3							++	NA	1-5	Water	F
Carbenicillin	C346	422.4	+	++						500	>1000	Water	F
Cefotaxime	C380	477.4	+	++						90	>100	Water	F
Chloramphenicol	C252	323.1	++	++	+			+	++	128	1-64	EtOH	R
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
Hygromycin B	H370	527.5							++	NA	20-400	Water	R
Kanamycin	K378	582.6	++	++				++	++	100	2	Water	R
Neomycin	N584	908.9	++	++					++	50	900	Water	R
Nystatin	N581	926.1				++	++			50	40	Not soluble	F
Paromomycin	P710	713.7	++						++		50	Water	R
Penicillin G	P777	356.4	++							Varies	100	Water	F
Rifampicin	R501	822.9	++	++	++					15	100	Water (Slight)	F
Spectinomycin	S742	405.3	+	++					++	20	500	Water	F
Streptomycin	S739	1457	++	++						100	16	Water	F
Tetracycline	T859	480.9	++	++						10	50	Water	F ³
Timentin	T869	NA		++					++		200	Water	F
Vancomycin	V870	1485	++							5	80	Water	R

++ = Effective against most microorganisms

+ = Effective against certain microorganisms

¹The 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.

²Solution storage: F = Freezer; R = Refrigerator; RT = Room Temperature

³Aqueous solutions of Tetracycline hydrolyze (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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