

Use of Antibiotics and Antimycotics

The decision to use antibiotics to prevent contamination should be based on the individual researcher's needs and experience. The following table is a general guide for use of GIBCO® antibiotics in tissue culture media. The concentrations given are for tissue culture media containing serum;

serum-free media generally require lower concentrations ($\frac{1}{2}$ to $\frac{1}{10}$). Also available are solutions that use one or more antibiotics in conjunction with an antimycotic. For all medium types, optimal concentrations of antibiotics and antimycotics should be determined empirically.

Antibiotic	Recommended Concentration	Antibiotic Spectrum	Stability in Tissue Culture Media at 37°C
Fungizone™ Reagent (Amphotericin B)	0.25–2.5 µg/ml	Fungi and yeasts	3 days
Gentamicin Sulfate	5–50 µg/ml	Gram positive and gram negative bacteria and mycoplasma	5 days
Kanamycin Sulfate	100 µg/ml	Gram positive and gram negative bacteria and mycoplasma	5 days
Neomycin Sulfate	50 µg/ml	Gram positive and gram negative bacteria	5 days
Penicillin G	50–100 units/ml	Gram positive bacteria	3 days
Polymixin B Sulfate	100 units/ml	Gram negative bacteria	5 days
Streptomycin Sulfate	50–100 µg/ml	Gram positive and gram negative bacteria	3 days

Decontaminating Cultures with Antibiotics and Antimycotics

When an irreplaceable culture becomes contaminated, researchers may attempt to eliminate or control the contamination. First, determine if the contamination is bacteria, fungus, mycoplasma, or yeast. Isolate the contaminated culture from other cell lines. Clean incubators and laminar flow hoods with a laboratory disinfectant, and check HEPA filters.

Antibiotics and antimycotics at high concentrations can be toxic to some cell lines; therefore, perform a dose-response test to determine the level at which an antibiotic or antimycotic becomes toxic. This is particularly important when using an antimycotic such as Fungizone™ Reagent or an antibiotic such as tylosin. The following is a suggested procedure for determining toxicity levels and decontaminating cultures.

1. Dissociate, count, and dilute the cells in antibiotic-free medium. Dilute the cells to the concentration used for regular cell passage.
2. Dispense the cell suspension into a multiwell culture plate or several small flasks. Add the antibiotic of choice to each well in a range of concentrations. For example, for Fungizone™ Reagent, 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 µg/ml.
3. Observe the cells daily for signs of toxicity such as sloughing, appearance of vacuoles, decrease in confluency, and rounding.
4. When the toxic antibiotic level has been determined, culture the cells for 2 to 3 passages using the antibiotic at a concentration 1- to 2-times lower than the toxic concentration.
5. Culture the cells for 1 passage in antibiotic-free medium.
6. Repeat step 4.
7. Culture the cells in antibiotic-free medium for 4 to 6 passages to determine if the contamination has been eliminated.

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