

## Antibiotic Treatments of a Microbe Contaminated Cell Culture

This protocol outlines the use of antibiotics to salvage a contaminated cell culture, but care must be taken not to overuse antibiotics. The overuse of antibiotics can result in antibiotic resistant microbes which can be of a health hazard to humans. If the contamination has not cleared after 14 days of treatment the culture should be destroyed.

<b>Antibiotics</b>					
<b>Organism</b>	<b>Antibiotic</b>	<b>Gold Biotechnology Catalog Number</b>	<b>Solvent</b>	<b>Stability (Days at 37 °C)</b>	<b>Working Concentration</b>
<b>Bacteria (gram-positive)</b>	Ampicillin	<a href="#">A0104</a>	Water	3	100 mg/L
	Erythromycin	<a href="#">E0122</a>	2 M HCl	3	100 mg/L
	Kanamycin sulfate	<a href="#">K0126</a>	Water	5	100 mg/L
	Neomycin sulfate	<a href="#">N0135</a>	Water	5	50 mg/L
	Streptomycin sulfate	<a href="#">S0148</a>	Water	3	100 mg/L
	Tetracycline HCl	<a href="#">T0150</a>	Water	4	10 mg/L
<b>Fungi</b>	Amphotericin B	<a href="#">A0103</a>	DMSO or DMF	3	2.5 mg/L
	Nystatin	<a href="#">N0138</a>	DMF	3	2.5x10 <sup>6</sup> U/L

1. As soon as contamination has been observed in a cell culture quarantine culture so that no other culture within the laboratory becomes contaminated.
2. Identify if the contaminant is bacteria or fungi
3. If the microbial contaminant can be identified choose an appropriate antibiotic
  - a. Prepare the antibiotic in the concentration noted on the table above. Be sure to filter sterilize the antibiotic after dissolved in the solvent noted.

4. If the bacterial contaminant is unknown prepare the following 10x antibiotic cocktail and treat cell culture:
  - 2500 U/mL Penicillin
  - 2.5 mg/mL Streptomycin sulfate
  - 2.5 mg/mL Neomycin
  - 25 U/mL Bacitracin ([Gold Biotechnology catalog number: B0106](#))

\*Be sure to filter sterilize cocktail
5. Centrifuge culture at 125g for 10 min
6. Remove supernatant and resuspend cells in fresh medium containing the antibiotics in the final working concentration noted
7. Add additional antibiotic solution every 3-5 days to maintain the working concentration, note the stability outlined in the table.
  - a. Passage of cells can be maintained as normal during this time, but care must be taken to maintain the antibiotic concentration in all originally contaminated samples.
8. Examine the contaminated culture microscopically during treatment. Be sure to look for cytotoxic effects as well as elimination of the contaminant.
9. If after 14 days contamination still exist destroy the culture by autoclaving.
10. If after 14 days contamination has been eliminated inoculate fresh antibiotic free media with the culture.

References:

Bonifacino et al. *Current Protocols in Cell Biology*. **2003**.