Troubleshooting Guide for Cell Culture Contamination

Common cell culture contaminants, their sources, detection, and prevention

CORNING

Type	Source	Detection	Prevention
Bacteria (Gram +, Gram -)	• Lab personnel • Unfiltered air • Humidified incubators • Purified water • Insects • Plants • Contaminated cell stock • Media • Equipment (Biosafety cabinets)	• Microbial culture • Gram's stain test • Visual turbidity • pH becomes acidic	• Aseptic technique • Antibiotics • Filtration (<0.22 µm) • Use of sterile products • Disinfection of CO_2 incubators • Daily cleaning of hood space with 70% alcohol, as well as monthly cleaning with 10% bleach or equivalent products • Daily, or at minimum weekly, emptying of used media traps • Storing cell line stocks in vapor phase of liquid nitrogen (LN_2), not in liquid phase
Yeast	• Humidified incubators • Lab personnel • Unfiltered air • Contaminated cell stock • Equipment (Biosafety cabinets)	• Microbial culture • Visual turbidity • Oder	• Aseptic techniques • Antimycotics • Filtration (<0.5 µm) • Use of sterile products • Disinfection of CO_2 incubators • Daily cleaning of hood space with 70% alcohol, as well as monthly cleaning with 10% bleach or equivalent products • Daily, or at minimum weekly, emptying of used media traps • Storing cell line stocks in vapor phase of LN_2, not in liquid phase
Fungus	• Fruit • Cellulose products (cardboard) • Plants • Unfiltered air • Lab personnel • Contaminated cell stock • Equipment (Biosafety cabinets)	• Microbial culture • Visual particulates, visual mycelia	• Aseptic technique • Antimycotics • Filtration (<0.5 µm) • Use of sterile products • Daily cleaning of hood space with 70% alcohol, as well as monthly cleaning with 10% bleach or equivalent products • Daily, or at minimum weekly, emptying of used media traps • Storing cell line stocks in vapor phase of LN_2, not in liquid phase
Virus	• Original tissues • Serum • Cross-contamination • Lab personnel • Equipment (Biosafety cabinets)	• Co-cultivation • PCR • Electron microscopy • *In vivo* testing • Assays	• Aseptic technique • Ultrafiltration • Chemical treatment • Gamma-irradiated serum • Use animal-free products • Storing cell line stocks in vapor phase of LN_2, not in liquid phase
Endotoxin	• Serum • Bacterial contamination • Contaminated or improperly maintained water supply • Equipment (Biosafety cabinets)	• Limulus amoebocyte lysate (LAL) assay	• Aseptic technique • Ultrafiltration (<5,000 daltons) • Endotoxin-specific affinity or size exclusion chromatography • Use animal-free products
Mycoplasma	• Contaminated cell lines • Serum • Media • Lab personnel • Equipment (Biosafety cabinets)	• Hoechst stain • Microbial culture • Specialized kits • PCR	• Aseptic technique • Antibiotics • Ultrafiltration (<0.04 µm) • Use of sterile products • Use animal-free products • Storing cell line stocks in vapor phase of LN_2, not in liquid phase
Cellular	• Cross-contamination of cultures • Cross-use or sharing of media from a different cell line • Equipment (Biosafety cabinets)	• Cell authentication to determine identity and species of cells	• Work with only one cell line at a time • Thoroughly clean before and after introducing a new cell line into the laminar flow hood • Daily cleaning of hood space with 70% alcohol, as well as monthly cleaning with 10% bleach or equivalent products • Storing cell line stocks in vapor phase of LN_2, not in liquid phase

NOTE: When applicable, resterilize media using a Corning® syringe filter or bottle-top filter.

Corning® media products used for the prevention of contamination:

Sterile Media and Reagents

Cat. No.	Description	Size	Qty/Pk
25-950-CQC	Dimethyl Sulfoxide (DMSO)	250 mL	1
35-070-CV	Gamma-irradiated FBS	500 mL	1

Prophylactic Antibiotics

Cat. No.	Description	Size	Qty/Pk
30-001-CI	Pen-Strep solution, 50X	100 mL	6
30-002-CI	Pen-Strep solution, 100X	100 mL	6
30-009-CI	Pen-Strep-L-Glutamine, 100X	100 mL	6
30-004-CI	Antibiotic-Antimycotic solution	100 mL	6
61-277-RF	Ciprofloxacin Hydrochloride, powder	1 g	1
61-277-RG	Ciprofloxacin Hydrochloride, powder	5 g	1

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

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At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

For additional product or technical information, please visit www.corning.com/lifesciences/media or call 1.800.235.5476.

Mediatech, Inc.
A Corning Subsidiary
9345 Discovery Boulevard
Manassas, VA 20109
t 800.235.5476
t 703.471.5955
f 703.467.9851
www.corning.com/lifesciences/media

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