CELL CULTURE CONTAMINATION

InvivoGen *in focus*

PRACTICAL GUIDE

OVERVIEW OF MICROBIAL CONTAMINANTS

Whether remaining unnoticed or expanding rapidly, microbes can seriously alter cell morphology and functions, becoming a serious threat to your research. InvivoGen offers microbial detection tools and elimination reagents, as well as preventive tips.

- Protect your cells
- Mycoplasma contaminations
- Bacterial contaminations
- Endotoxin contaminations
- Fungal contaminations

InvivoGen *infocus*

A PRACTICAL GUIDE TO AVOID CELL CULTURE CONTAMINATION

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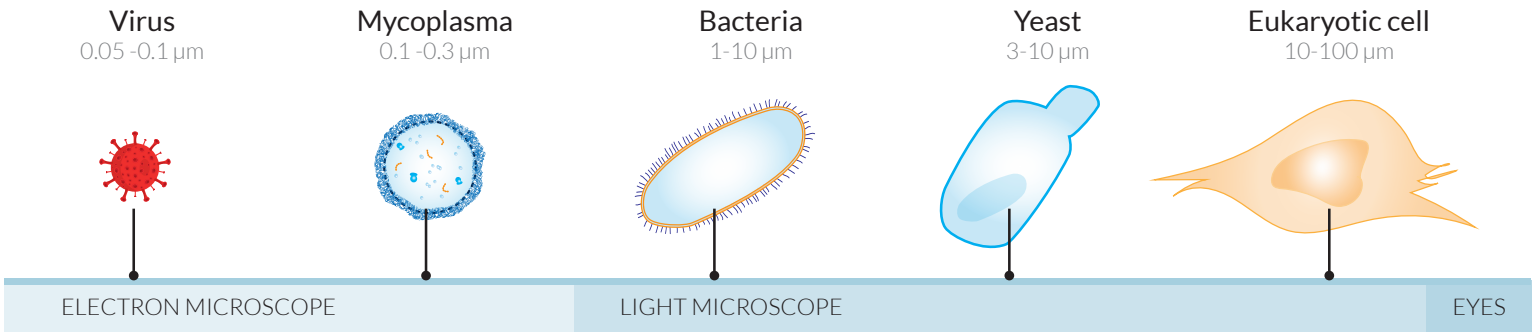
Microbial contamination of cell cultures is a serious and relentless threat to your research. Invasive mycoplasma, bacteria, and fungi can kill or drastically alter cells in culture, leading to disastrous results, lost time, and wasted resources. This brochure provides an insight into the contaminants that are most likely to invade your cultures, the good practices to avoid them, and the solutions to eliminate them. As experts in innate immunity and microbiology, we know how these biological contaminants can interfere with experimental results. While bacterial and fungal contaminations are eventually detected by the naked eye, mycoplasma and endotoxins remain invisible. Undetected contaminants are a serious concern, as they may have led to data misinterpretation, many of which have been published. As a consequence, journals now frequently ask for evidence of absence of mycoplasma and endotoxins in cell cultures. Moreover, pharmaceutical companies developing future therapeutics cannot afford contaminations as they will compromise their research and reputation. At InvivoGen, we strive for excellence. We provide high-quality products and mycoplasma-free cell lines all around the world. This guide will help you address every stage of microbial infection, and choose the right InvivoGen product to detect, eliminate, and prevent contaminations in your cell cultures.

ABBREVIATIONS

CFU : colony-forming unit
DNA : deoxyribonucleic acid
IRF : interferon regulatory factor
kDa : kilodalton
LAL : limulus amebocyte lysate
LPS : lipopolysaccharide
NF-κB : nuclear factor kappa-light-chain enhancer of activated B cells
PCR : polymerase chain reaction
PRR : pattern recognition receptor
rRNA : ribosomal ribonucleic acid
SEAP : secreted embryonic alkaline phosphatase
TLRs : toll-like receptors

TINY ORGANISMS, BIG HEADACHES



1956

First detection and isolation of mycoplasma in cell cultures

+180

Different mycoplasma species have been identified

AT LEAST 20

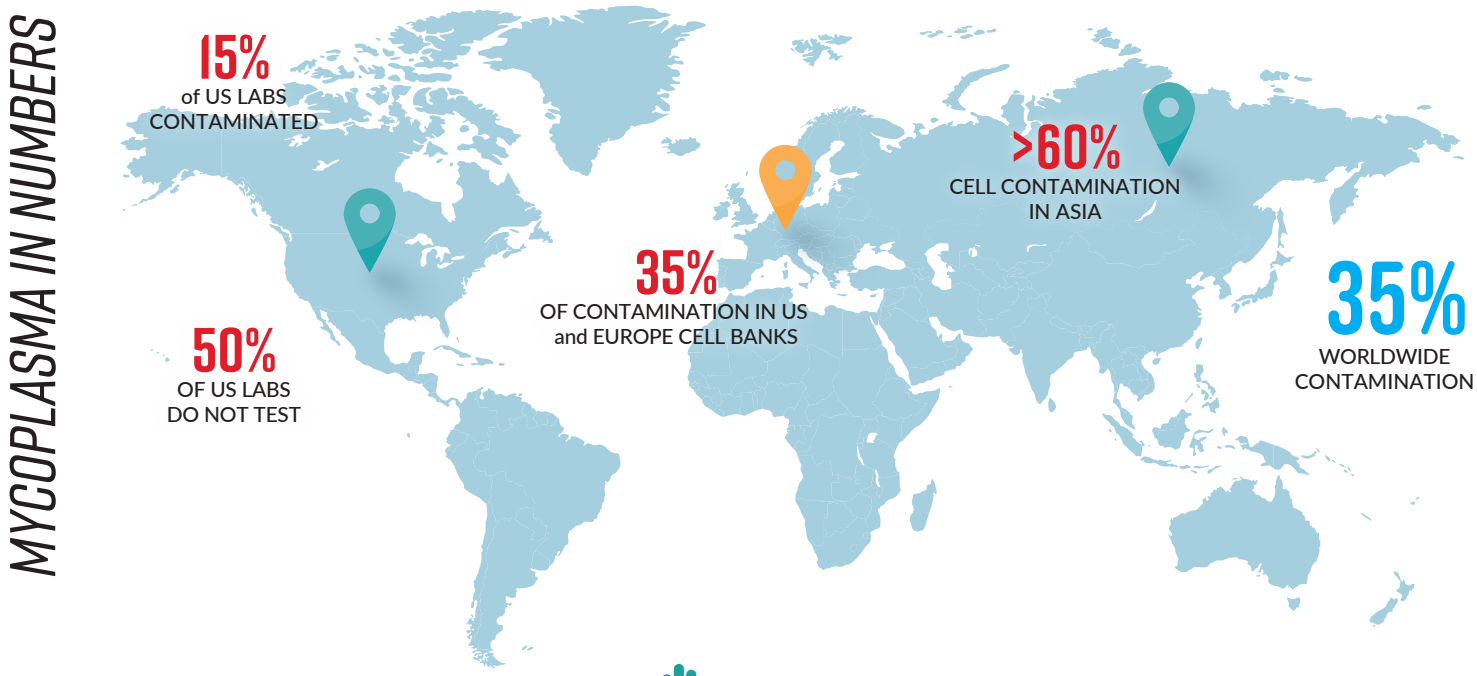
Distinct mycoplasma species isolated from contaminated cell cultures

95%

of cell culture contamination is due to 6 mycoplasma species

- *M. orale*
- *M. fermentans*
- *M. hyorhinis*
- *M. hominis*
- *M. arginini*
- *A. laidlawii*

MYCOPLASMA IN NUMBERS



80% OF LAB OPERATORS CARRY MYCOPLASMA

Mycoplasma contamination testing status must also be reported.

It is good to obtain cell lines from reputable repositories, to routinely authenticate cell line stocks and test them for mycoplasma contamination

CONTAMINATION PREVENTION TIPS

- *Quarantine all new cell cultures and animal products entering the laboratory*
- *Be vigilant and always practice good aseptic technique*
- *Avoid talking over your cells*
  6% of operators spread mycoplasma by talking
- *Avoid sneezing*
  38% of operators spread mycoplasma by sneezing
- *Routinely test your cells*
- *Ask manufacturers for certification proving the absence of mycoplasma contamination*

YOUR CELLS ARE PRECIOUS, PROTECT THEM!

Detection

Microbial contamination must be detected as early as possible. Detection methods depend on the nature of the microbe. They include biological assays, PCR, fluorescence or chemical staining, optical microscopy, turbidimetry, pH measurements, or simple visual inspection. Bacteria and fungi can usually be identified by optical microscopy. Their fast growth rate allows their detection by the naked eye as early as 48 hours (i.e. over the weekend), the contaminated cultures appearing turbid or spotty. Subsequently, identification of these micro-organisms can be performed with testing kits. Mycoplasma in cell cultures cannot be detected visually, not even by optical microscopy. Hence, these microbes can go unnoticed for long periods and are identified using dedicated assays.

Prevention

Knowing the sources of microbial contamination is crucial for minimizing the risk to cell cultures (see below). Although absolute prevention is impossible, you can take various measures to prevent infection. Firstly, ensure that you are working in a sterile environment and using proper aseptic technique. Secondly, quarantine any incoming cell cultures until these have been confirmed free of contamination. Thirdly, monitor your cell cultures for contamination on a regular basis by optical microscopy and detection kits. Lastly, you can use antibiotic cocktails, such as those offered by InvivoGen, specifically designed for taking a preventive strike against microbes that would be difficult to detect in new cultures (i.e. primary cells, or cloning).

Elimination

Typically, once invasive microbes are detected in cell cultures it is recommended to discard the cells and the media. However, some cell cultures are so precious that they cannot be lost (i.e. stable clone selection, cell lines derived from explanted tissues, primary cells) and are not available elsewhere (i.e. not yet frozen). In such situations, InvivoGen provides antibiotics to eradicate the contamination surely and rapidly without damaging your cells.

COMMON SOURCES OF MICROBIAL CONTAMINATION IN THE LABORATORY

1. INFRASTRUCTURE:
Fume-hoods, ventilation units and laboratory furniture can house surface microorganisms and spread airborne ones. Clean working areas daily with alcohol and monthly with bleach. Regularly change all air filters, and empty all media traps at least weekly.

2. OPERATOR:
Laboratory staff can transmit microbes and dust from their skin, clothes and bodies to cell cultures. Wear proper safety garments and use aseptic technique.

3. PIPETTES, TIPS, SYRINGES & VACUUM PUMPS:
A contaminated pipette can destroy multiple cell cultures. Use sterile pipettes, tips and syringes and never reuse disposables. Make sure to empty and clean the vacuum pump reservoir and tubings regularly.

4. EQUIPMENT:
Glassware, incubators and water baths can easily be contaminated. Keep all equipment sterile and frequently change the water in the baths, which are notorious for fungal infections. Regularly disinfect all incubators.

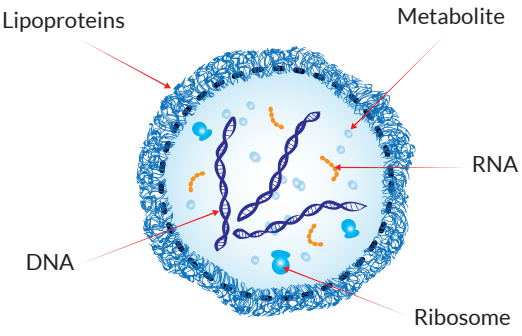
5. SAFETY GARMENTS:
Labcoats and gloves must be clean and should be worn only in the lab. Use disposable garments only once and discard them immediately afterwards.

6. CULTURE MEDIA, REAGENTS & CELLS:
Before using any culture medium, reagent or cells, confirm that they are sterile. Carefully seal all containers.

MYCOPLASMA CONTAMINATIONS

Mycoplasma are the smallest and simplest self-replicating organisms. Because of their small size (100 nm) and lack of a rigid cell wall, mycoplasma are undetectable by visual inspection, pass through standard filtration, and are resistant to a large number of antibiotics[1]. Mycoplasma contamination is a major problem in cell culture, affecting the validity of experimental results as well as the quality and safety of cell-based biopharmaceuticals[2].

Mycoplasma features

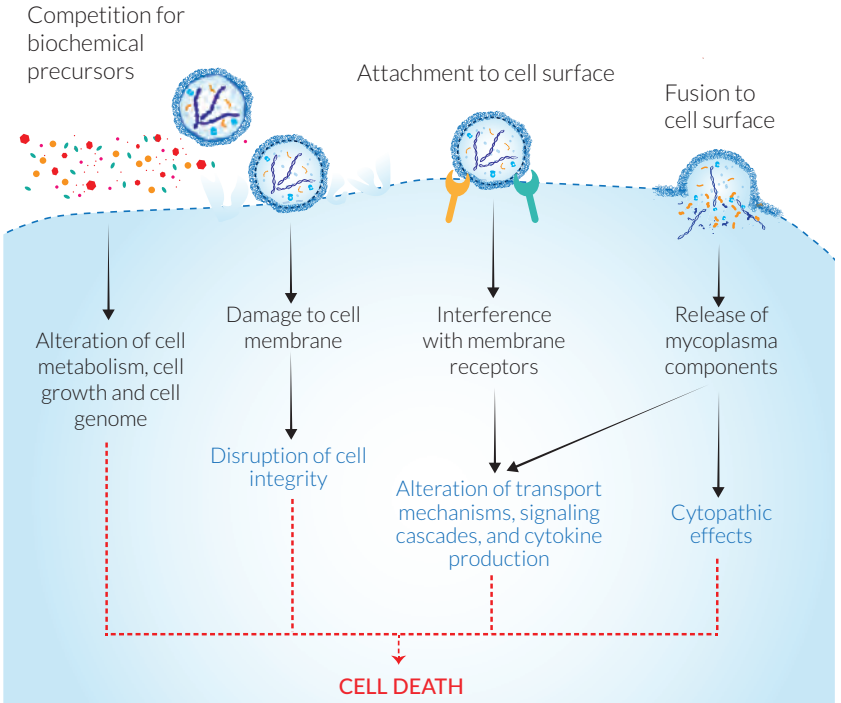


Mycoplasma belong to the class of Mollicutes, which members are distinguished by their lack of cell wall and their plasma-like form. Mycoplasma are highly infectious, for all types of eukaryotic cells, including primary cells. Hundreds of mycoplasma can attach to a single cell, fuse with the cell membrane, multiply, and eventually outnumber cultured cells by 1000-fold. Mycoplasma can drastically alter cell cultures and skew research results (see below). Mycoplasma lipoproteins are potent activators of immune cells upon sensing by Toll-like receptor 2 (TLR2), their preferential pattern recognition receptor (PRR)[3]. The absence of a cell wall in mycoplasma confers them resistance to commonly used antibiotics, such as penicillin and streptomycin. Moreover, their tiny size (~100 nm) does not allow their elimination by standard 0.2 µm filtration. Thus, major precautions are required to prevent contamination of cell cultures.

All InvivoGen's cell lines are guaranteed mycoplasma-free based on PlasmoTest™ results

Major impacts of mycoplasma contamination on cell functions

Mycoplasma compete with host cells for nutrients and biochemical precursors. As a consequence, they alter many cell functions, such as cell metabolism and cell growth, ultimately leading to cell death. A microarray analysis on contaminated cultured human cells has revealed the severe effects that mycoplasma can have on the expression of hundreds of genes, including some that encode receptors, ion channels, growth factors, and oncogenes[4]. Upon adhesion or fusion with the host cell membrane, they can cause further damage to the cell by interfering with signaling cascades and cytokine production[5]. These detrimental effects can strongly impact scientific results and invalidate the findings of a study, especially when it involves immune cells expressing TLR2, such as macrophages[3,6].



TOP 5 REASONS TO TEST

- **Loss of precious cell lines**
- **Serious impact on data reliability and reproducibility**
- **Testing is required by most journals for publication**
- **Up to 35% worldwide contamination, incuding cell banks**
- **Loss of time and money**

DETECTION OF MYCOPLASMA CONTAMINATION

InvivoGen offers two mycoplasma detection kits to allow timely intervention. Both kits allow fast and accurate detection of mycoplasma species that most commonly contaminate cell culture. MycoStrip™ is based on 'immediate' (~1 h) detection of mycoplasma genomic content using immunochromatographic strips. PlasmoTest™ is a colorimetric cellular assay based on detection of mycoplasma lipoproteins.

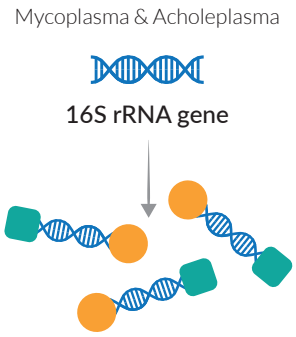
Catch mycoplasma off-guard using 'immediate' and 'routine' tests

Genomic detection strips

NEW

MycoStrip™

- **Recommended for immediate results**
- **Simple:** No special lab equipment required
- **Rapid:** Hands-on time <15 min. Total duration: 1 h.
- **Clear:** One band – negative for mycoplasma
  Two bands – positive for mycoplasma



Isothermal PCR

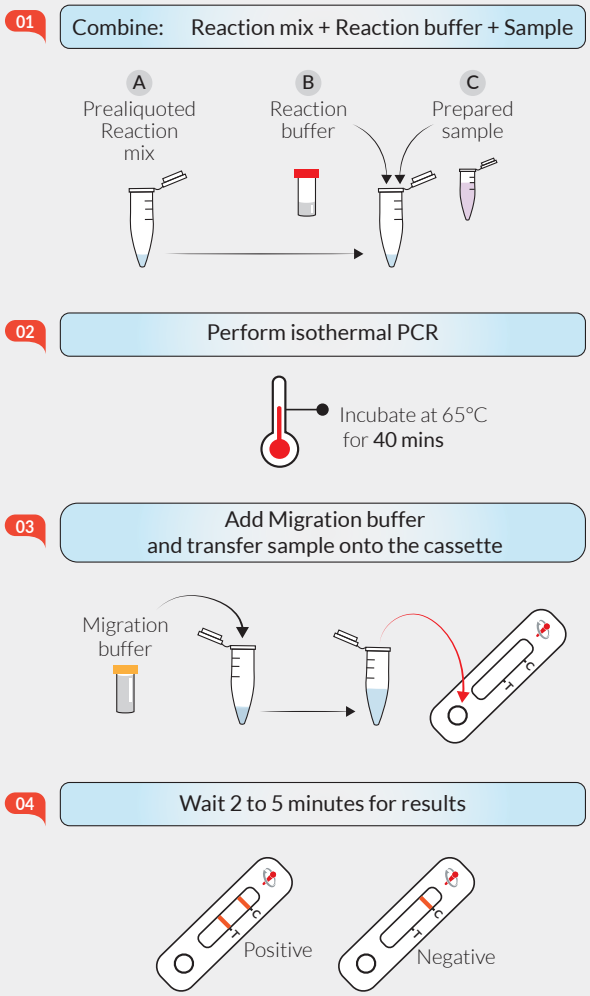
Immunochromatographic strip

Detection of cell culture contaminating mycoplasma by MycoStrip™ is based on isothermal PCR. The 16S rRNA gene for the most commonly found mycoplasma species in cell culture, accounting for 95% of contamination, is targeted and amplified using our proprietary Reaction mix. Results are visualized as a band on an immunochromatographic strip within 5 minutes.

PRODUCT	DESCRIPTION	QTY	CAT. CODE
MycoStrip™	Mycoplasma contamination detection kit (strips)	10 tests 20 tests 50 tests 100 tests*	rep-mys-10 rep-mys-20 rep-mys-50 rep-mys-100

*without cassette

MycoStrip™ procedure



FREQUENTLY ASKED QUESTIONS

Q: Do MycoStrip™ and PlasmoTest™ detect only live mycoplasma?

A: Both kits detect live and dead mycoplasma. MycoStrip™ and PlasmoTest™ detect the presence of mycoplasma DNA and lipoproteins, respectively.

Q: Does PlasmoTest™ detect only mycoplasma in cell cultures?

A: PlasmoTest™ relies on the activation of TLR2. Therefore, it can detect both mycoplasma and bacteria contaminants. However, while the mycoplasma cannot be detected by the naked eye, bacteria contamination is visible and leads to a decreased pH (change of medium color to yellow) and medium turbidity due to bacterial growth.

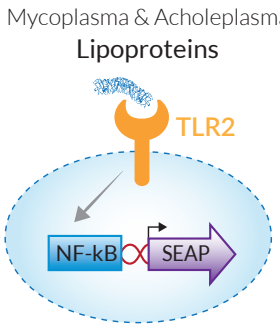
More FAQs online

www.invivogen/mycostrip & www.invivogen/plasmotest

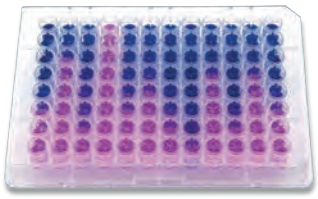
Colorimetric cellular assay

PlasmoTest™

- **Recommended for routine tests**
- **Simple:** Colorimetric detection in cell culture supernatant
- **Rapid:** Hands-on time <1 h. Results overnight.
- **Sensitive:** LOD 5.10^2 - 5.10^5 CFU/ml of culture supernatant



Reporter cell line

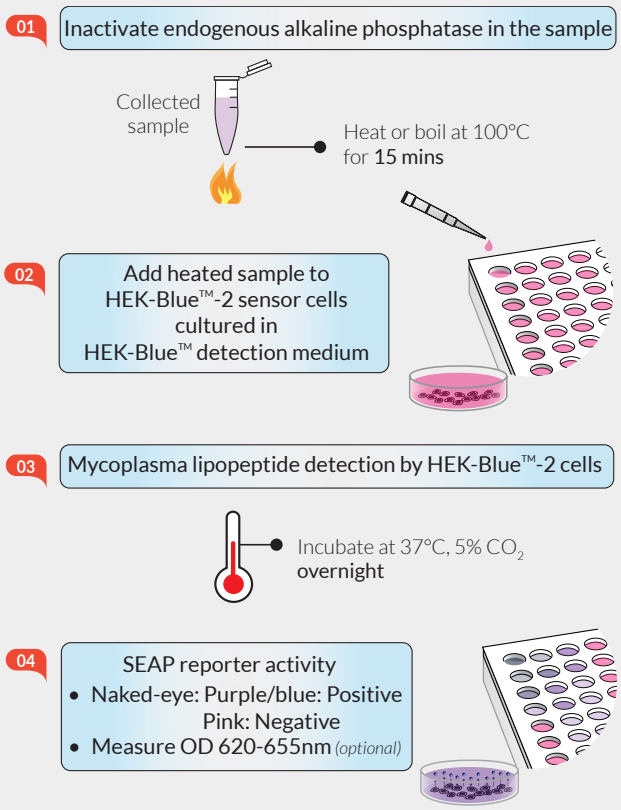


Purple/blue: Positive
Pink: Negative

Colorimetric read-out

PlasmoTest™ relies on Toll-like receptor 2 (TLR2), the preferential pattern recognition receptor (PRR) for mycoplasma lipoproteins[1]. Our proprietary HEK-Blue™-2 cells stably express human TLR2 and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. They are cultured in HEK-Blue™ detection medium, make them ideal for routine tests. The simple addition of test samples to these cells provides colorimetric results with sensitivity similar to luminescence-based biochemical assays. SEAP activity can be measured at 620-655 nm using a spectrophotometer. The absorbance is in direct proportion to the amount of contaminants. For your convenience, HEK-Blue™-2 cells are provided with Normocin™, an antibiotic cocktail to prevent cell culture contamination with mycoplasma, bacteria, and fungi (*see page 11*).

PlasmoTest™ procedure



MYCOPLASMA DETECTION METHODS

	MycoStrip™	PlasmoTest™
Target	16S rRNA gene	Lipoproteins
Method	Isothermal PCR	Reporter bioassay
Ease of use	++++	++
Specificity	At least the 8 most common species	
Sensitivity	10-10^2 CFU/ml	10^2-10^5 CFU/ml
Experiment duration	<1 hr	<1 hr (hands on) OVN (incubation)
Additional equipements/ reagents	Heating bath/block	Incubator Spectrophotometer
Naked-eye data visualization	Yes	Yes
Cost	Reasonable	Cost-effective

WHAT IF MY TEST IS POSITIVE ?

Your culture is easily treatable with InvivoGen's anti-mycoplasma reagents.

Treat your culture and eradicate the contamination using Plasmocin™ or Plasmocure™. Upon completion of the treatment (~2 weeks), re-test using MycoStrip™ or PlasmoTest™ comparing your newly treated culture with your previous sample .

PRODUCT	DESCRIPTION	QTY	CAT. CODE
PlasmoTest™	Mycoplasma contamination detection kit (cells)	1 kit (250 tests)	rep-pt1
PlasmoTest™ Controls	Controls for PlasmoTest™ detection kit	200 tests	pt-ctr2
PlasmoTest™ Refills	Reagents for PlasmoTest™ detection kit	500 samples	rep-ptrk

ELIMINATION OF MYCOPLASMA

InvivoGen has over 40 years of experience in developing anti-mycoplasma solutions for the scientific community. Plasmocin™ and Plasmocure™ are unique anti-mycoplasma reagents that combine two distinct sets of antibiotics in a single ready-to-use product. They act fast with little to no cytotoxicity, ensuring you save precious cell lines and data.

Plasmocin™ and Plasmocure™ allow broad and rapid elimination of mycoplasma contaminants.

A preventive & removal treatment

Plasmocin™

- **Recommended for broad mycoplasma elimination**
- **Fast:** Rescues cell cultures in 2 weeks
- **Safe:** Little to no toxicity on mammalian cells
- **2 formats:** Prophylactic and Treatment

Plasmocin™, a frequently cited mycoplasma removal agent[7-11], is effective against all common mycoplasma strains, both extracellular and intracellular. For maximum efficiency, Plasmocin™ contains a formulation of two antibiotics: the first one blocks protein synthesis, and the second one stops DNA replication. A component of Plasmocin™ is actively transported into mammalian cells, ensuring that following treatment, cell cultures do not become re-infected. Thus, Plasmocin™ is more effective than other reagents on the market in eradicating mycoplasma and preventing resistant strain generation[7].

- **Plasmocin™ prophylactic** is a product that can be used on a regular basis to prevent mycoplasma contaminations.
- **Plasmocin™ treatment** is intended for mycoplasma elimination within 2 weeks.

An alternative removal treatment

Plasmocure™

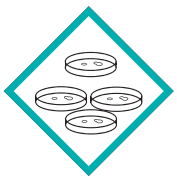
- **Recommended for Plasmocin™-resistant strains**
- **Fast:** Rescues cell cultures in 2 weeks
- **Safe:** Little to no toxicity on mammalian cells
- **Reliable:** Extremely low mycoplasma regrowth rate

Plasmocure™ is a second-line anti-mycoplasma reagent that potently eradicates Plasmocin™-resistant mycoplasma[12]. It combines two antibiotics that act through different mechanisms than those found in Plasmocin™. The first antibiotic binds to the 50S subunit of the ribosome and blocks peptidyltransferase activity. The second antibiotic binds to isoleucyl-tRNA synthetase and halts isoleucine incorporation into mycoplasma proteins.
A two-week treatment with Plasmocure™ is usually sufficient to completely eliminate mycoplasma. If mycoplasma elimination is not completed after two weeks, Plasmocure™ can be administered for an additional week. A moderate slowdown of cell growth may be observed, but full recovery of the cell line is expected once mycoplasma are eliminated.

Tips for successful mycoplasma elimination

OPTIMAL TREATMENT CONCENTRATION

We recommend to test 3 different treatment concentrations according to the protocol, including a no-treatment condition.



BACK-UP CELLS

Maintain a culture duplicate and/or frozen vial without treatment:
• to validate the treatment efficacy
• to start over with new treatment conditions if necessary.

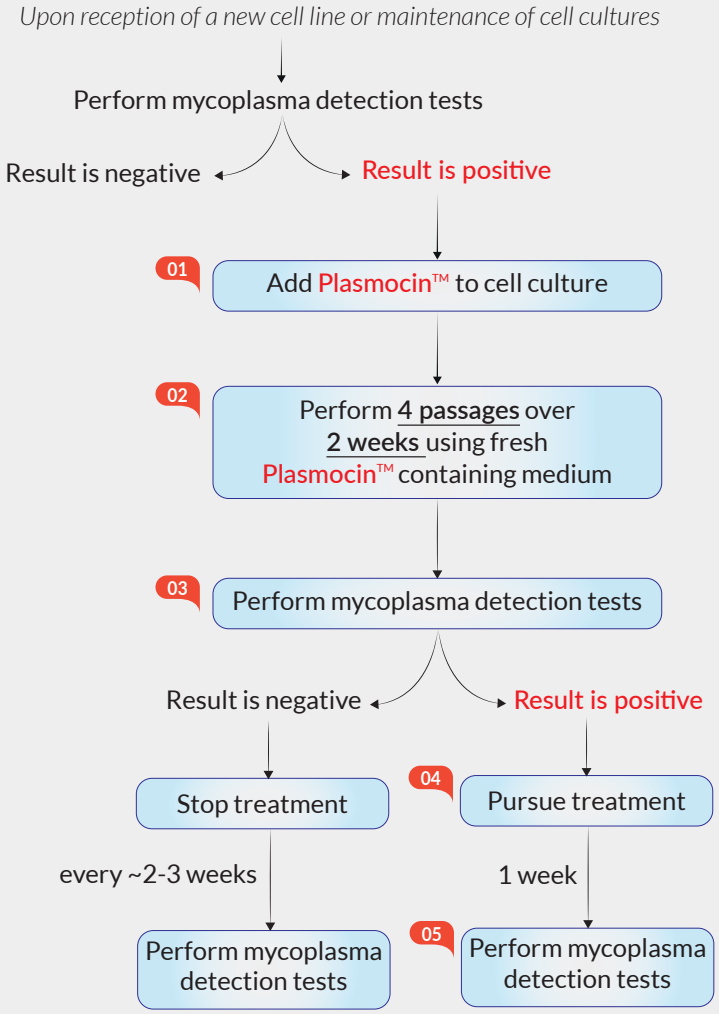
ALTERNATIVE TREATMENT

In case of a lack of Plasmocin™ efficacy, use Plasmocure™ (or *vice versa*).

REGULAR TESTING

Early mycoplasma detection, using MycoStrip™ or PlasmoTest™, allows timely intervention. We recommend testing cell cultures every ~2-3 weeks.

Mycoplasma surveillance and elimination



Use Plasmocure™ for Plasmocin™-resistant mycoplasma contaminations

MYCOPLASMA ELIMINATION REAGENTS

	Plasmocin™	Plasmocure™
Treatment duration	2 weeks	2 weeks
Ease of use	++++	++++
Efficacy	+++	++++
Cytotoxicity	+/-	+/-
Resistance[11]	-	- -

FREQUENTLY ASKED QUESTIONS

Q: Can I use Plasmocin™ during the initial culture phase before making my frozen stocks?

A: Yes, it is even recommended. However, it is necessary to check that the cells are not contaminated using MycoStrip™ or PlasmoTest™.

Q: What is the toxicity of Plasmocin™ and Plasmocure™ to eukaryotic cells?

A: Plasmocin™ and Plasmocure™ targets are absent in eukaryotic cells, ensuring low cytotoxicty.

Q: What to use? Plasmocin™ or Plasmocure™?

A: If your cells are positive for mycoplasma, we recommend starting a treatment with Plasmocin™. Plasmocure™ should be used in the case of resistance to Plasmocin™.

More FAQs online

www.invivogen/plasmocin & www.invivogen/plasmocure

DON'T STRESS

Many infected cell lines have been successfully treated with Plasmocin™

Including cancer cell lines[9], virus-producing cells[10], induced pluripotent stem cells[11], and human embryonic stem cells[13] with no permanent alterations.

They trust InvivoGen

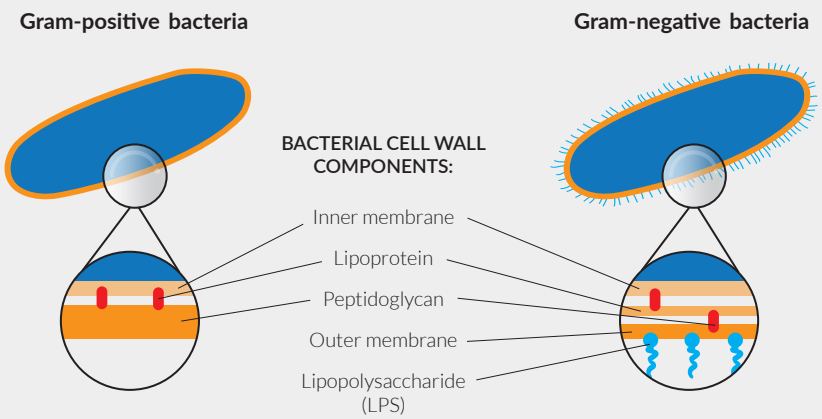
— Kapralov A. A. *et al.*, 2020. *Nat Chem Biol*. Redox lipid reprogramming commands susceptibility of macrophages and microglia to ferroptotic death. DOI: 10.1038/s41589-019-0462-8.
— Kazemiha V. M. *et al.*, 2019. *Cell J.* Effectiveness of Plasmocure™ to eliminate mycoplasma species from contaminated cell cultures: A comparative study versus other antibiotics. DOI: 10.22074/cellj.2019.5996

PRODUCT	DESCRIPTION	QTY	CAT. CODE
Plasmocin™ prophylactic	Reagent for preventing mycoplasma contamination	25 mg (10 x 1 ml)	ant-mpp
Plasmocin™ treatment	Mycoplasma elimination reagent	25 mg (1 x 1 ml) 50 mg (2 x 1 ml)	ant-mpt-1 ant-mpt
Plasmocure™	Mycoplasma elimination reagent	100 mg (1 ml)	ant-pc

BACTERIAL CONTAMINATIONS

Bacteria are a large and ubiquitous group of unicellular micro-organisms. They are typically a few micrometers in diameter and can have a variety of shapes, ranging from spheres to rods and spirals. Bacteria are the most commonly encountered biological contaminants in cell culture[14, 15]. Despite being detectable using a light microscope, bacteria can easily be mistaken for cellular debris, especially during the early stages of contamination.

The usual bacterial suspects



Examples*:
Staphylococcus epidermidis
Bacillus subtilis

Examples*:
Pseudomonas aeruginosa
Klebsiella enterobacter
Flavobacterium spp.
Escherichia coli
Achromobacter spp.
Alcaligenes spp.
Bordetella spp.

*see references [16-19]

> Bacteria can easily be mistaken for cellular debris, especially at the early stages of contamination.

Detection of bacterial contamination

In the early stages of contamination, bacteria can be mistaken for cellular debris as they are much smaller (1-10 µm) than eukaryotic cells (10-100 µm). Therefore, it is important to check your cell cultures under a light microscope using phase contrast (100x - 400x).
Do you see an abnormal presence of small black dots, rods, spirals, either alone, in chains, or clusters? Are they motile? If so, your culture is probably contaminated.

Because of their fast growth rate, bacteria cause a change in the culture medium in just 48 hours, making the contamination clearly visible with the naked eye from 10^5 CFU/ml. The culture medium appears cloudy, and if it contains phenol red, a rapid color change from red to yellow indicates a decrease in pH, a consequence of bacteria metabolism. The culture environment is no longer suitable for eukaryotic cells leading eventually to their death.
These cultures should be discarded, or if irreplaceable, treated with InvivoGen's antibiotic cocktails *(see next page)*.

Sources of contamination

LAB OPERATORS
Major source of contamination with *Staphylococcus* species.

DIRTY WATER BATHS, INCUBATORS, GLASSWARE, ...
Major source of contamination with *Pseudomonas* and *Flavobacterium* species.

CELLS ISOLATED FROM ANIMAL TISSUES
Contamination from the commensal flora and/or subclinical infections.

ELIMINATION OF BACTERIA

Contamination preventive reagent

Normocin™

- **Broad-spectrum:** Kills mycoplasma, bacteria, and fungi
- **Safe:** Little to no toxicity on mammalian cells

Normocin™ is an innovative formulation of three antibiotics active against mycoplasma, bacteria (Gram+ and Gram-), and fungi, including yeasts. It is widely used and cited as a "routine addition" to cell culture media to prevent contamination. Normocin™ can be used in combination with penicillin and streptomycin (Pen-Strep) solutions to broaden the anti-bacterial spectrum. Normocin™ provides maximum protection against microbial contamination with minimum cytotoxicity.

DID YOU KNOW?

All InvivoGen's cell lines are provided with Normocin™, a broad-spectrum anti-microbial agent, to help you keep your cells safe

Anti-microbial agent for primary cells

Primocin™

- **Broad-spectrum:** Kills mycoplasma, bacteria, and fungi
- **Safe:** Little to no toxicity on mammalian cells

Primocin™ contains four compounds, with three of these blocking DNA and protein synthesis in Gram+ bacteria, Gram- bacteria, and mycoplasma. The fourth compound eradicates fungi, including yeasts, by disrupting ionic exchange through the cell membrane. Primocin™ has been successfully used with many primary cells, including mouse- and human-tumor-derived cell lines, embryonic cells, and induced pluripotent stem cells.

Multidrug-resistant bacteria removal agent

Normocure™

- **Ready-to-use:** Add directly into medium bottles or flasks
- **Fast:** Rescues cell cultures in 3 passages
- **Safe:** Little to no toxicity on mammalian cells

Normocure™ is the best weapon to save your valuable cell lines from Gram+ and Gram- bacteria, especially non-fermenting Gram- bacteria that are resistant to Pen-Strep and Normocin™. Normocure™ is a cocktail of three components belonging to different antibiotic families. After the first passage, >99% of bacterial contaminants are eliminated. The targets of these antibiotics are absent in eukaryotic cells, ensuring Normocure™'s low cytotoxicity.

FREQUENTLY ASKED QUESTIONS

Q: Do the anti-microbial agents you have in your catalog interfere with selective antibiotics?

A: No, they do not interfere with common selective antibiotics such as G418, Blasticidin, Puromycin, Hygromycin B, or Zeocin™.

Q: We have a bacterial contamination but cannot determine which bacterial strain has contaminated our cultures. What would be the best option to ensure bacteria elimination?

A: We would highly recommend using Normocure™ which is a broad-spectrum antibacterial agent highly effective against Gram+ and Gram- bacteria. Cell cultures contaminated with bacteria from the environment, such as Staphylococcus species and Achromobacter species, can be efficiently cured by Normocure™ treatment.

More FAQ online

www.invivogen/normocin, www.invivogen/normocure , www.invivogen/primocin

They trust InvivoGen

— Magupalli V. G. *et al.*, 2020. *Science*. HDAC6 mediates an aggresome-like mechanism for NLRP3 and pyrin inflammasome activation. DOI: 10.1126/science.aas8995.
— Polyzoz A. A. *et al.*, 2019. *Cell Metab.* Metabolic Reprogramming in Astrocytes Distinguishes Region-Specific Neuronal Susceptibility in Huntington Mice. DOI: 10.1016/j.cmet.2019.03.004.
— Ganesh K. *et al.*, 2020. *Nat. Cancer.* L1CAM defines the regenerative origin of metastasis-initiating cells in colorectal cancer. DOI: 10.1038/s43018-019-0006-x.

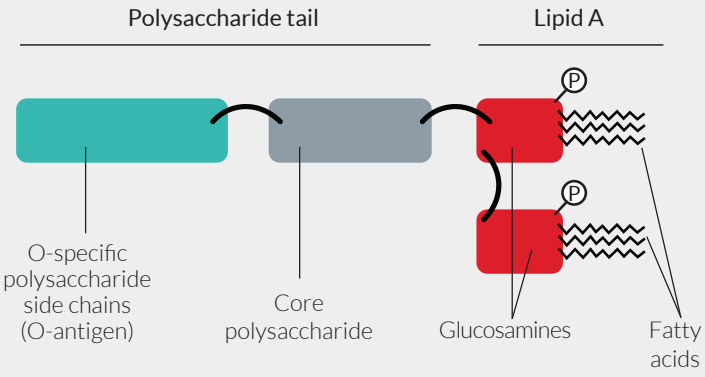
PRODUCT	DESCRIPTION	QTY	CAT. CODE
Normocin™	Reagent for preventing microbial contamination	500 mg (10 x 1 ml) 1 g (1 x 20 ml)	ant-nr-1 ant-nr-2
Normocure™	Microbial contamination elimination reagent	100 mg (2 x 1 ml)	ant-noc
Primocin™	Reagent for preventing microbial contamination in primary cells	500 mg (10 x 1 ml) 1 g (1 x 20 ml)	ant-pm-1 ant-pm-2

ENDOTOXIN CONTAMINATIONS

Endotoxins, also known as lipopolysaccharides (LPS) or lipoglycans, are a major cell wall component of Gram-negative bacteria. Sources of endotoxins include media, sera, water, buffers, and other cell culture reagents, such as trypsin. Endotoxins are potent inducers of inflammatory responses both *in vitro* and *in vivo*. Extra care needs to be taken with solutions and reagents that are sterile but may still contain bacterial components, and monitoring for the presence of endotoxins in cell culture reagents is crucial.

Endotoxin contaminations are a major concern for cell cultures and production of injectable drugs.

Endotoxin features



- 3 - 40 kDa: depending on the size of the O-antigen
- Amphiphilic: >1000 kDa aggregates in aqueous solution
- UV-resistant and thermally-stable (up to 180°C)
- Polysaccharide tail: size varies among Gram-negative bacteria species. Confers specific antigenic responses in humans.
- Lipid A: confers LPS toxicity. Stimulates the mammalian immune system.

Sources of contamination

WATER
Use high-purity water to prepare media and solutions, and clean glassware.

GLASSWARE
To destroy endotoxins, heat glassware to 250°C for≥30 min, or 180°C for 3 hrs.

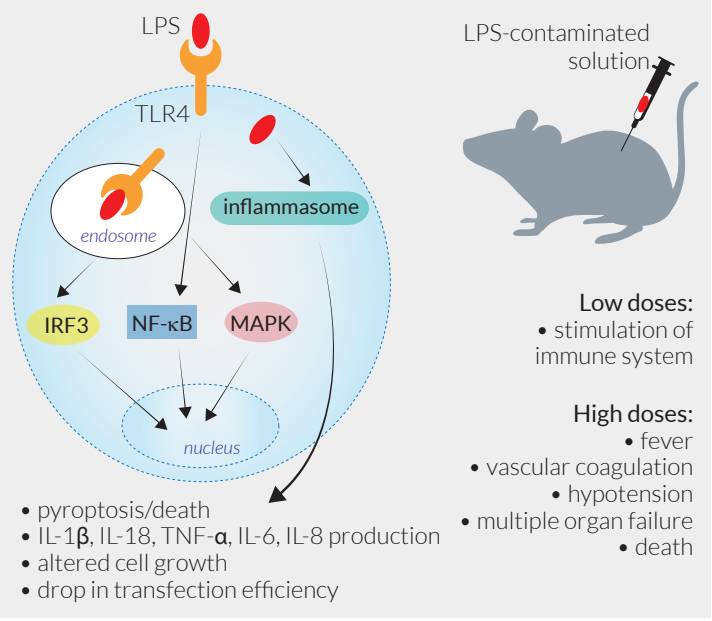
MEDIA, SERA & ADDITIVES
Ask for manufacturer certification of endotoxin levels, or test before use.

PLASTICWARE
Ask for manufacturer certification of endotoxin levels and absence of pyrogenicity.

Risks for *in vitro* and *in vivo* experiments

The lipid A moiety of LPS activates the Toll-like receptor 4 (TLR4) at the cell surface or in endosomes, and subsequently induces the activation of MAP Kinases, NF-κB, and IRF (interferon regulatory factor) pathways[20, 21]. Although the effects of endotoxins vary according to concentration and cell type, it has been shown that these molecules can alter cellular morphology, proliferation, and transfection efficiency[22]. Moreover, LPS induces the production of inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-8, and IL-18, and activation of the caspase 4/5/11-NLRP3 non-canonical inflammasome and cause pyroptotic cell death[20, 23].

In vivo, low endotoxin concentrations can stimulate the immune system, while high concentrations can induce fever, hypotension, multiple organ failure, and even death[22].



DETECTION OF ENDOTOXINS IN BIOLOGICAL REAGENTS

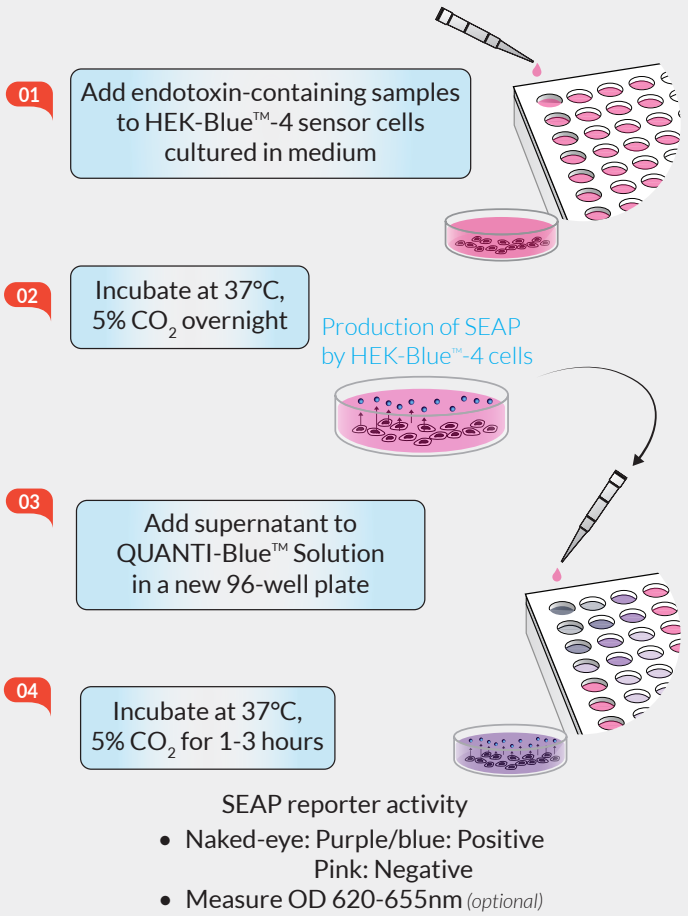
Colorimetric cellular assay

HEK-Blue™ LPS Detection Kit 2

- **Versatile:** Detection in virtually all biological reagents
- **Highly sensitive:** Detects as little as 0.01 EU/ml
- **Economical:** 1 kit allows to perform up to 500 tests

This kit relies on InvivoGen's proprietary HEK-Blue™-4 cells, which stably express human TLR4 and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. The simple experimental procedure makes this kit ideal for routine tests. The presence of minute quantities of LPS in test samples incubated overnight with the HEK-Blue™-4 cells leads to the activation of NF-κB and expression of the SEAP reporter. The activity of SEAP in the culture supernatant is assessed by QUANTI-Blue™ Solution, a colorimetric SEAP detection medium, and measured at 620-655 nm using a spectrophotometer. The absorbance is in direct proportion to the amount of endotoxin present. The endotoxin concentration can be calculated from a standard curve obtained using serial dilutions of the HEK-Blue™ Endotoxin Standard provided in the kit.

Principle of LPS Detection Kit 2



SEAP reporter activity

- Naked-eye: Purple/blue: Positive
  Pink: Negative
- Measure OD 620-655nm *(optional)*

TOP 5 REASONS TO USE

- **Sustainable alternative to the costly and laborious limulus amebocyte lysate (LAL) assay.** Freeze HEK-Blue™-4 cells and re-order the other components separately.
- Unlike the LAL assay, HEK-Blue™-4 cell-based LPS detection **does not lead to false positives** if the sample contains (1,3)-β-D-glucan.
- **For all biological samples**, including medium, serum, chemical preparations, and vaccine adjuvants.
- **Convenient visualization of results by change of medium color** from pink to purple/blue.
- **Includes a booklet for experimental procedures, a graphical method to calculate the endotoxin concentration, and a troubleshooting section.**

They trust InvivoGen

— Gray M. A. *et al.*, 2020. *Nat Chem Biol*. Targeted glycan degradation potentiates the anticancer immune response in vivo. DOI: 10.1038/s41589-020-0622-x.
— Imbert P. R. C. *et al.*, 2021. *Curr Biol.* An Acquired and Endogenous Glycocalyx Forms a Bidirectional "Don't Eat" and "Don't Eat Me" Barrier to Phagocytosis. DOI: 10.1016/j.cub.2020.09.082.

PRODUCT	DESCRIPTION	QTY	CAT. CODE
HEK-Blue™ LPS Detection Kit 2	Assay for the detection and quantification of biologically active LPS	1 kit	rep-lps2
HEK-Blue™ Selection	Antibiotics for maintenance of HEK-Blue™ cells	10 x 1 ml	hb-sel
HEK-Blue™ Endotoxin Standard	Standardized *E. coli* 055:B5 LPS	10 x 50 EU	rep-hbes-10
QUANTI-Blue™ Solution	Alkaline phophatase detection medium (liquid form, 100X)	5 ml 10 ml	rep-qbs rep-qbs2

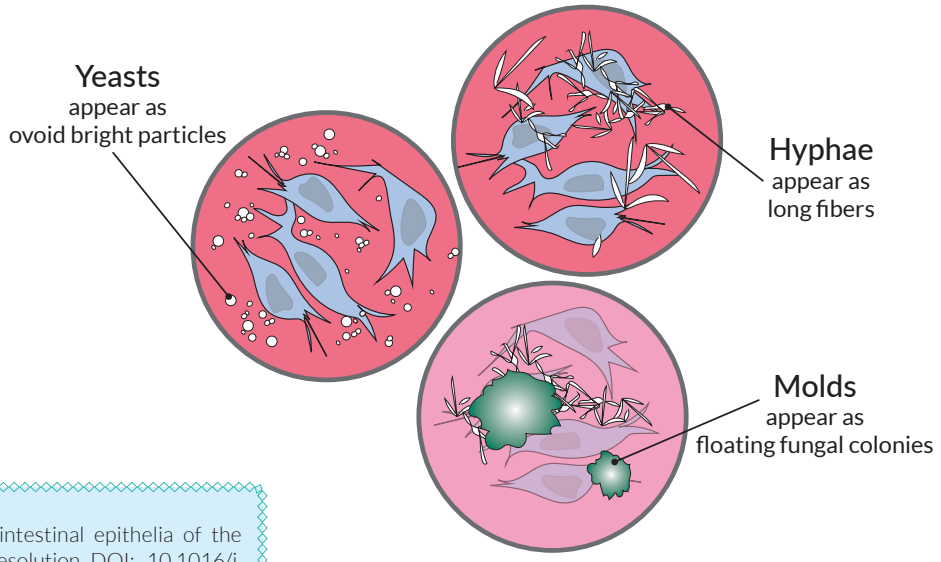
FUNGAL CONTAMINATIONS

Unlike mycoplasma and bacteria, fungi are eukaryotes and can exist as round or oval bodies (yeasts) that can form chains or clusters, or as long thin filaments (hyphae)[24]. Molds are a group of hyphae that appear as fuzzy patches in the advanced stages of contamination[24]. Fungal contamination is a major problem in cell culture, affecting the validity of experimental results. More importantly, these types of contaminations are extremely hard to eradicate, as fungi can spread via spore mobility in the air. Dormant spores of many fungal species can survive in extremely harsh and inhospitable environments, only to become activated when they encounter suitable growth conditions.

Cell cultures can often be cured of fungal contamination when detected early and treated with Fungin™ antimycotic agent.

How to detect fungal contamination in cell cultures?

Yeasts are the smallest form (3-10 µm) of fungi. They can be seen using a light microscope.
Hyphae can be detected by the naked eye or a light microscope depending on their size and growth stage.
In the case of substantial contamination, colonies form as molds floating on the surface. **In this case, do not open the vessel and discard the cultures to avoid spreading spores.** Sometimes, the medium pH may increase, resulting in phenol-red-containing media appearing pink.



They trust InvivoGen

— Busslinger G.A. *et al.*, 2021. *Cell Rep.* Human gastrointestinal epithelia of the esophagus, stomach, and duodenum resolved at single-cell resolution. DOI: 10.1016/j.celrep.2021.108819
— Lebreton F. *et al.*, 2019. *Nat Commun.* Insulin-producing organoids engineered from islet and amniotic epithelial cells to treat diabetes. DOI: 10.1038/s41467-019-12472-3.

ELIMINATION OF FUNGI

For preventive and removal treatments

Fungin™

- **Effective:** Kills fungi (yeasts, hyphae, and molds)
- **Fast:** Rescues cell cultures in 5-10 days
- **Safe:** Minimum toxicity on mammalian cells
- **Reliable:** Can be used as a prophylactic treatment

Fungin™ is a soluble antimycotic compound that kills the different forms of fungi (yeasts, hyphae, and molds) by disrupting ionic exchange through the cell membrane. It is a highly stable compound and does not need to be dissolved in deoxycholate (which is cytotoxic). Therefore, it is an excellent alternative to the use of Amphotericin B antimycotic. Fungin™ can be used at either low or high concentrations for routine prevention or contamination removal, respectively. It provides maximum protection against fungal contaminants commonly found in cell culture, such as *Candida albicans* and *Aspergillus spp.* Fungin™ may be added to media containing commonly used antibacterial agents, such as penicillin and streptomycin (Pen-Strep). Fungin™ is a highly referenced antimycotic reagent.

PRODUCT	DESCRIPTION	QTY	CAT. CODE
Fungin™	Reagent for preventing fungal contamination	75 mg (5 x 1.5 ml) 200 mg (1 x 20 ml)	ant-fn-1 ant-fn-2

SUMMARY TABLE OF INVIVOGEN'S ANTIMICROBIAL AGENTS

CONTAMINANT	PRODUCT	DESCRIPTION	PAGE
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	PlasmoTest™	Mycoplasma contamination detection kit	7
	PlasmoTest™ Controls	Controls for PlasmoTest™ detection kit	7
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	Plasmocin™ prophylactic	Reagent for preventing mycoplasma contamination	8
	Plasmocin™ treatment	Mycoplasma elimination reagent	8
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Bacteria	Normocin™	Reagent for preventing microbial contamination	11
	Normocure™	Microbial contamination elimination reagent	11
	Primocin™	Reagent for preventing microbial contamination in primary cells	11
Endotoxins	HEK-Blue™ LPS Detection Kit 2	Assay for the detection and quantification of biologically active LPS	13
	HEK-Blue™ Selection	Antibiotics for maintenance of HEK-Blue™ cells	13
	HEK-Blue™ Endotoxin Standard	Standardized *E. coli* 055:B5 LPS	13
	QUANTI-Blue™ Solution	Alkaline phophatase detection medium (liquid form)	13
Fungi	Fungin™	Reagent for preventing and eliminating fungal contamination	14

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MycoStrip™ Mycoplasma Detection Kit

A new way to detect mycoplasma in your cell culture

- ***Simple*** *- No special equipment required*
- ***Rapid*** *- Performed in 1 hour, less than 15 min of hands-on time*
- ***Clear*** *- One band: negative, Two bands: positive for Mycoplasma*
- ***Specific*** *- No cross reactity with eukaryotic or other bacterial DNA*
- ***Sensitive*** *- Able to detect as low as 10-10^2 CFU/ml*

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