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
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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CFU</td>
<td>colony-forming unit</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>IRF</td>
<td>interferon regulatory factor</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>LAL</td>
<td>limulus amebocyte lysate</td>
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<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor kappa-light-chain enhancer of activated B cells</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PRR</td>
<td>pattern recognition receptor</td>
</tr>
<tr>
<td>rRNA</td>
<td>ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>SEAP</td>
<td>secreted embryonic alkaline phosphatase</td>
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<tr>
<td>TLRs</td>
<td>toll-like receptors</td>
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</tbody>
</table>
MYCOPLASMA IN NUMBERS

First detection and isolation of mycoplasma in cell cultures

Different mycoplasma species have been identified

Distinct mycoplasma species isolated from contaminated cell cultures

MYCOPLASMA IN NUMBERS

Virus
0.05 - 0.1 µm

Mycoplasma
0.1 - 0.3 µm

Bacteria
1 - 10 µm

Yeast
3 - 10 µm

Eukaryotic cell
10 - 100 µm

ELECTRON MICROSCOPE
LIGHT MICROSCOPE
EYES

1956

180

AT LEAST 20

95%

15% of US LABS CONTAMINATED

50% OF US LABS DO NOT TEST

80% OF LAB OPERATORS CARRY MYCOPLASMA

MYCOPLASMA IN NUMBERS

35% OF CONTAMINATION IN US and EUROPE CELL BANKS

>60% CELL CONTAMINATION IN ASIA

35% WORLDWIDE CONTAMINATION

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

InvivoGen OFFERS

>14 ANTI-MICROBIAL SPECIFIC REAGENTS & ASSAYS TO ACCOMPANY YOUR SUCCESSFUL RESEARCH
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.

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.

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).

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 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. 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.

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). 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.

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. 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. 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.
DETECTION OF MYCOPLASMA CONTAMINATION

In vivoGen 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.

Genomic detection strips

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

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.

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>DESCRIPTION</th>
<th>QTY</th>
<th>CAT. CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MycoStrip™</td>
<td>Mycoplasma contamination detection kit (strips)</td>
<td>10 tests</td>
<td>rep-mys-10</td>
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<td></td>
<td></td>
<td>20 tests</td>
<td>rep-mys-20</td>
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<tr>
<td></td>
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<td>50 tests</td>
<td>rep-mys-50</td>
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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/plasmo-test
**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

PlasmoTest™ relies on Toll-like receptor 2 (TLR2), the preferential pattern recognition receptor (PRR) for *mycoplasma lipoproteins*. 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**

1. **Inactivate endogenous alkaline phosphatase in the sample**
   - Heat or boil at 100°C for 15 mins
2. **Add heated sample to HEK-Blue™-2 sensor cells cultured in HEK-Blue™ detection medium**
3. **Incubate at 37°C, 5% CO2 overnight**
4. **SEAP reporter activity**
   - Naked-eye: Purple/blue: Positive
   - Pink: Negative
   - Measure OD 620-655nm (optional)

**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.

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**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-prtk
**ELIMINATION OF MYCOPLASMA**

nvivoGen 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.

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, 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.

- **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. 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.
**PRODUCT DESCRIPTION**

**QTY**

**CAT. CODE**

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>DESCRIPTION</th>
<th>QTY</th>
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<tr>
<td>Plasmocin™ prophylactic</td>
<td>Reagent for preventing mycoplasma contamination</td>
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<td>ant-mpp</td>
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<td>Plasmocin™ treatment</td>
<td>Mycoplasma elimination reagent</td>
<td>25 mg (1 x 1 ml)</td>
<td>ant-mpt-1</td>
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<tr>
<td></td>
<td></td>
<td>50 mg (2 x 1 ml)</td>
<td>ant-mpt</td>
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<tr>
<td>Plasmocure™</td>
<td>Mycoplasma elimination reagent</td>
<td>100 mg (1 ml)</td>
<td>ant-pc</td>
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</tbody>
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**MYCOPLASMA ELIMINATION REAGENTS**

<table>
<thead>
<tr>
<th></th>
<th>Plasmocin™</th>
<th>Plasmocure™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment duration</td>
<td>2 weeks</td>
<td>2 weeks</td>
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<tr>
<td>Ease of use</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Efficacy</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Resistance</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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**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 cytotoxicity.

**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

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**DON'T STRESS**

Many infected cell lines have been successfully treated with Plasmocin™

- Including cancer cell lines
- Virus-producing cells
- Induced pluripotent stem cells
- Human embryonic stem cells with no permanent alterations.

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They trust InvivoGen

**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\textsuperscript{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

<table>
<thead>
<tr>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
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</thead>
<tbody>
<tr>
<td><strong>BACTERIAL CELL WALL COMPONENTS:</strong></td>
<td><strong>BACTERIAL CELL WALL COMPONENTS:</strong></td>
</tr>
<tr>
<td>Inner membrane</td>
<td>Inner membrane</td>
</tr>
<tr>
<td>Lipoprotein</td>
<td>Lipopolysaccharide (LPS)</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>Outer membrane</td>
</tr>
<tr>
<td>Outer membrane</td>
<td></td>
</tr>
<tr>
<td>Lipopolysaccharide (LPS)</td>
<td></td>
</tr>
</tbody>
</table>

**Examples*:**
- Staphylococcus epidermidis
- Bacillus subtilis

*see references 16-19

### 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.

### 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).
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-negative 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-negative 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

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

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. aaz9995.
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 features

- **Lipid A**: confers LPS toxicity. Stimulates the mammalian immune system.
- **O-specific polysaccharide side-chains (O-antigen)**
- **Core polysaccharide**
- **Glucosamines**
- **Fatty acids**
- **UV-resistant and thermally-stable** (up to 180°C)
- **Amphiphilic**: >1000 kDa aggregates in aqueous solution
- **3 - 40 kDa**: depending on the size of the O-antigen

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. 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. 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.

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

**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.

**PRODUCT DESCRIPTION**

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<thead>
<tr>
<th>PRODUCT</th>
<th>DESCRIPTION</th>
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</thead>
<tbody>
<tr>
<td>HEK-Blue™ LPS Detection Kit 2</td>
<td>Assay for the detection and quantification of biologically active LPS</td>
<td>1 kit</td>
<td>rep-lps2</td>
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<tr>
<td>HEK-Blue™ Selection</td>
<td>Antibiotics for maintenance of HEK-Blue™ cells</td>
<td>10 x 1 ml</td>
<td>hb-sel</td>
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<td>HEK-Blue™ Endotoxin Standard</td>
<td>Standardized E. coli O55:B5 LPS</td>
<td>10 x 50 EU</td>
<td>rep-hbes-10</td>
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<td>QUANTI-Blue™ Solution</td>
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<tr>
<td></td>
<td></td>
<td>10 ml</td>
<td>rep-qbs2</td>
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</table>

**Principle of LPS Detection Kit 2**

1. **Add endotoxin-containing samples to HEK-Blue™-4 sensor cells cultured in medium**
2. **Incubate at 37°C, 5% CO₂ overnight**
3. **Add supernatant to QUANTI-Blue™ Solution in a new 96-well plate**
4. **Incubate at 37°C, 5% CO₂ for 1-3 hours**

**SEAP reporter activity**

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

**They trust InvivoGen**

**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). Molds are a group of hyphae that appear as fuzzy patches in the advanced stages of contamination. 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.

**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.

**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 contaminant species 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.

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>DESCRIPTION</th>
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<td>Fungin™</td>
<td>Reagent for preventing fungal contamination</td>
<td>75 mg (5 x 1.5 ml), 200 mg (1 x 20 ml)</td>
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</tbody>
</table>


They trust InvivoGen
REFERENCES


**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 reactivity with eukaryotic or other bacterial DNA
- **Sensitive** - Able to detect as low as 10^{-10} CFU/ml