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InvivoGen Insight

An Insightful Look At InvivoGen's Innovative Products

Mycoplasma contamination remains a significant problem to the culture of mammalian cells. The detection of mycoplasma contamination is an important part of mycoplasma control and should be an established method in every cell culture laboratory. InvivoGen is pleased to introduce PlasmoTest™, a Mycoplasma detection kit based on a brand new technology. PlasmoTest™ is sensitive, reliable and appropriately simple and economical for routine use. Cell cultures found contaminated can be efficiently treated by Plasmocin™, a common recourse for mycoplasma infection and one of InvivoGen's best sellers.

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Mycoplasma: The Insidious Invader of Cell Cultures

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Mycoplasma: The Insidious Invader of Cell Cultures

Mycoplasmas are the smallest and simplest self-replicating organisms. Due to their seriously degraded genome they cannot perform many metabolic functions, such as cell wall production or synthesis of nucleotides and amino acids. Mycoplasmas are strictly parasites. They parasitize a wide range of organisms including humans, animals, insects, and plants.

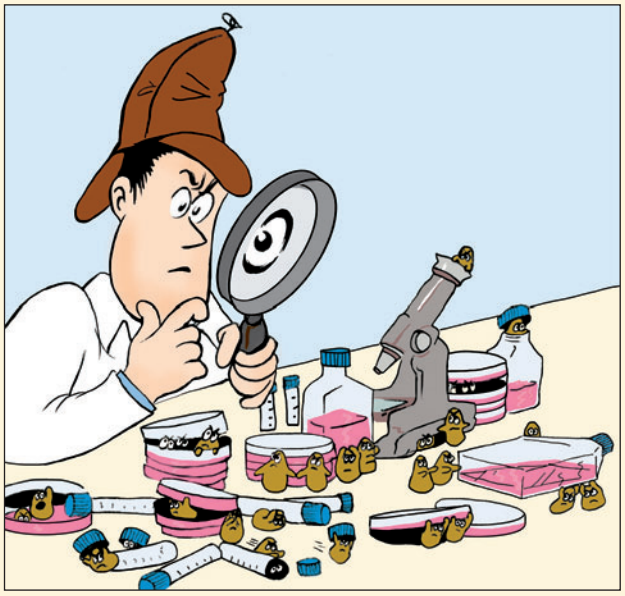
Mycoplasma and *Acholeplasma* are *Mollicutes*, that comprise together more than 100 recognized species. Among them, about 20 species have been described as contaminants of eukaryotic cell cultures. However 5 species (*Mycoplasma* (*M.*) *arginini*, *M. fermentans*, *M. orale*, *M. hyorhinis* and *Acholeplasma laidlawii*) are isolated in 90-95% of contaminated cell cultures[1].

Mycoplasma contamination of cell cultures occurs through 3 major sources: 1) incoming infected cells, 2) the laboratory personnel, potential carriers of *M. orale, M. fermentans, M. salivarium* or *M. hominis*, and 3) the reagents used in cell culture, such as bovine sera (*M. arginini, A. laidlawii*) or porcine trypsin (*M. hyorhinis*). Once a contamination is established, bacteria spread by aerosol droplet dispersion. If "Good Laboratory Practices" are not strictly maintained such as regular disinfection, all the equipment (pipettes, laminar flow hoods, incubators) can be contaminated and participate to the spread of mycoplasmal contamination[2].

In many cases there are no signs of mycoplasma contamination. In contrast to other microbial contaminants, mycoplasmas do not cause consistent perceptible changes in a cell culture, e.g. rapid pH change or culture turbidity[2,4]. They can grow to very high concentrations, typically 10^6-10^8/ml, but due to their small size (0.1-0.8 µm in diameter) they remain undetectable by microscopic observation. Although invisible, mycoplasmas can cause disastrous effects on eukaryotic cells as they can alter every cellular parameter from proliferation to virus susceptibility and production. The only way to confirm mycoplasma contamination is by routine testing using special techniques.

There are numerous methods for the detection of mycoplasmas among these are direct growth on broth/agar, DNA staining, PCR, ELISA, RNA labeling and enzymatic procedures. However, none of these methods is 100% reliable. Direct growth methods are relatively sensitive to most species but the overall procedure is lengthy (3 weeks), costly and less sensitive to noncultivable species. The PCR method, although rather fast and inexpensive, is limited by its sensitivity and the risk of positive and false negative results.

InvivoGen has developed a new mycoplasma detection method that promises to resolve these issues. This method is based on the detection of mycoplasmas by engineered cells that express Toll-like receptor 2, a pathogen recognition receptor that detects mycoplasmas. PlasmoTest™, InvivoGen's new mycoplasma detection kit, exploits this method. The kit contains all the reagents necessary to perform the assay. PlasmoTest™ is simple and affordable allowing its routine use for presumptive results.



1. McGarrity G. *et al*., 1992. Mycoplasmas and tissue culture cells. In: Maniloff, J., McElhaney, R.N., Finch, L.R., Baseman, J.B. (Eds.), Mycoplasmas, Molecular Biology and Pathogenesis, American Society for Microbiology, Washington DC, pp.445-54.

2. Lincoln CK,Gabridge MG., 1998. Cell culture contamination: sources, consequences, prevention, and elimination. Methods Cell Biol. 57: 49-65.

3. Razin S. *et al*., 1998. Molecular biology and pathogenicity of mycoplasma. Microbiol Mol Biol Rev. 62: 1094-1156.

4. Doyle A, Griffiths JB., 1998. The cell: selection and standardization. In: Cell and tissue culture: laboratory procedures in biotechnology. Doyle, A and Griffiths JB, Wiley and Sons, Ltd.35-52.

PlasmoTest™

HEK-Blue™ Mycoplasma Detection Kit

InvivoGen is pleased to introduce PlasmoTest™, a breakthrough in mycoplasma detection for cell cultures. PlasmoTest™ represents an entirely new method to detect the presence of mycoplasma contaminants in cell cultures. PlasmoTest™ is the first mycoplasma detection kit that uses engineered cells and therefore can be easily established as a routine procedure in the lab.

Principle

PlasmoTest™ is a cell-based colorimetric assay that exploits the ability of Toll-like receptor 2 to recognize mycoplasmas and to induce a signaling cascade leading to the activation of NF-κB and other transcription factors. In the presence of mycoplasmas, TLR2 expressed on the surface of **HEK-Blue™-2 cells** activates these transcription factors which in turn induce the secretion of sAP (secreted alkaline phosphatase), a reporter protein easily detectable by the purple/blue coloration of the **HEK-Blue™ Detection** medium.

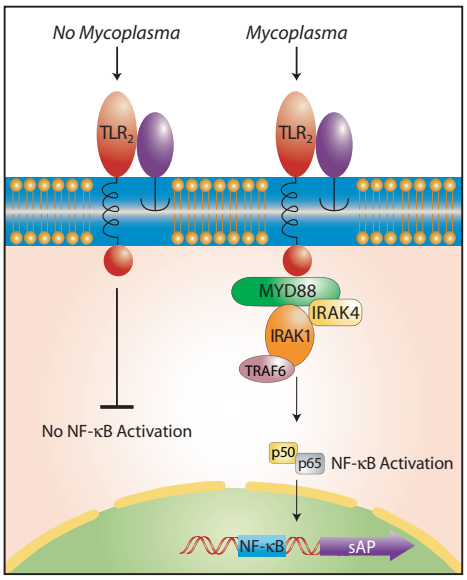


Figure 1: Principle of PlasmoTest™, a TLR2 activation based *Mycoplasma* Detection Kit.

- **Simple** - Requires only basic cell culture knowledge - No need for specific lab equipment.
- **Convenient** - Results are easily determined with the naked eye or quantified spectrophotometrically.
- **Rapid** - Hands-on time less than 1 hour - Gives results after overnight incubation.
- **Universal** - Detects all *Mycoplasma* and *Acheloplasma* species known to infect cell cultures.
- **Versatile** - Can Detect other cell culture contaminants such as bacteria.
- **Sensitive** - Detects 5.10^{2}-5.10^{5} cfu/ml mycoplasmas.
- **Reliable** - No false positive - A positive result indicates the presence of a cell culture contaminant.
- **Complete** - Contains the HEK-Blue™-2 cells and all the reagents needed to perform the assay, including positive and negative controls.
- **Economical** - Up to 500 samples can be tested with the kit - To perform further assays, only the reagents need to be reordered.

Key Features

• **HEK-Blue™-2 cells** are engineered HEK293 cells stably transfected with multiple genes from the TLR2 pathway that include TLR2 and genes participating in the recognition or involved in the signaling cascade. In addition, HEK-Blue™-2 cells stably express an optimized alkaline phosphatase gene engineered to be secreted (sAP), placed under the control of a promoter inducible by several transcription factors such NF-κB and AP-1 (figure 1).

HEK-Blue™-2 Selection is a solution that combines several selective antibiotics. These antibiotics guarantee the persistent expression of the various transgenes introduced in HEK-Blue™-2 cells. Furthermore, Normocin™ is included in the kit to protect HEK-Blue™-2 cells from any potential microbial contamination, whether caused by mycoplasmas, bacteria or fungi.

HEK-Blue™ Detection is a medium specifically designed for the detection of sAP. In the presence of mycoplasma-contaminated samples, HEK-Blue™-2 cells secrete sAP in the HEK-Blue™ Detection medium resulting in a color change from pink to purple/blue (figure 2).

Most common *Mycoplasma* detected

- *Acholeplasma laidlawii*
- *Acholeplasma vituli*
- *Mycoplasma arginini*
- *Mycoplasma fermentans*
- *Mycoplasma hominis*
- *Mycoplasma hyorhinis*
- *Mycoplasma orale*
- *Mycoplasma pirum*
- *Mycoplasma salivarium*

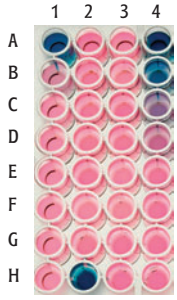


Figure 2: Visualization of *Mycoplasma* contaminations using HEK-Blue™-2 cells and HEK-Blue™ Detection medium.

	Controls	Cell line supernatants		Infected 293PR
A	Positive control	293/hTLR8	B16-F1	Direct
B	Positive control 1/10	293/hTLR5-HA	PC1-0	10^{-1}
C	Negative control	293/null	MEF	2.10^{-2}
D	Negative control	HEK293	MEF-IL12	10^{-2}
E	Negative control	HeLa	THP1	2.10^{-3}
F	Negative control	CHO-K1	Jurkat	10^{-3}
G	Negative control	C2C12	Cell culture medium	2.10^{-4}
H	Negative control	293PR (contaminated)	Cell culture medium	10^{-4}

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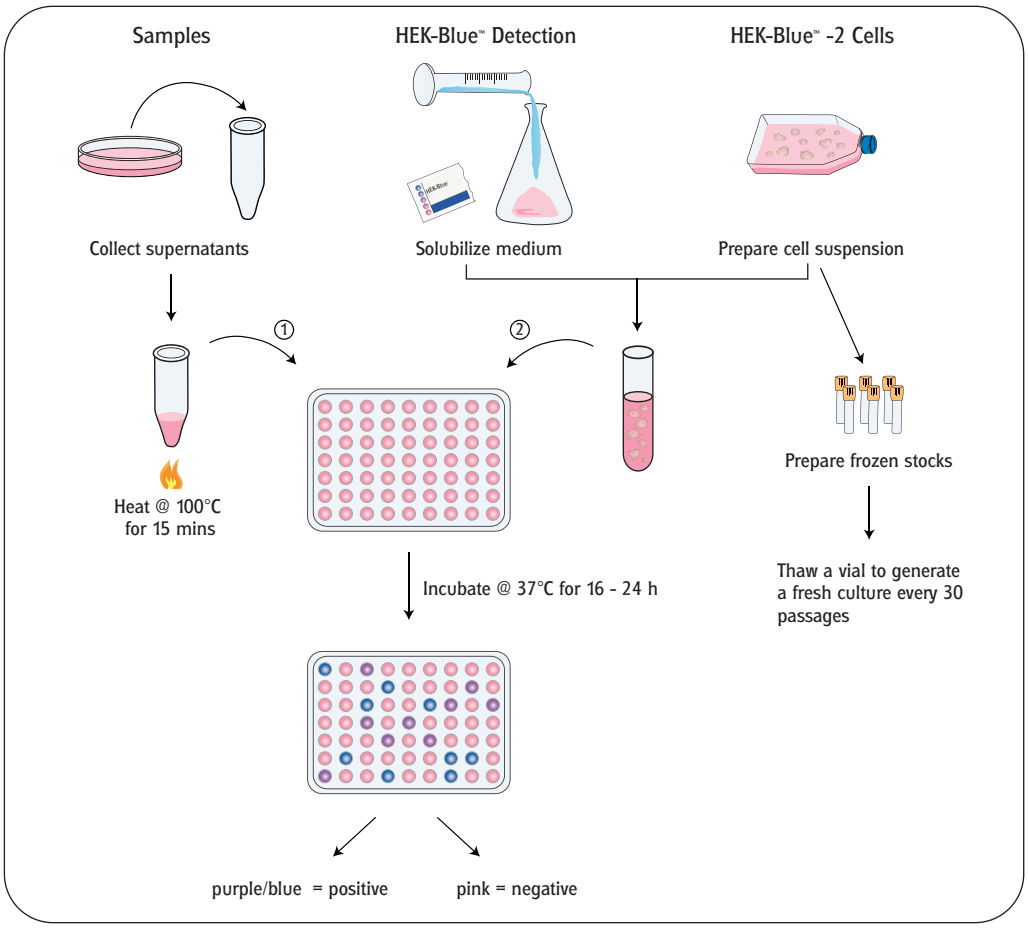
PlasmoTest™ Procedure



1- Collect 500 µl cell culture supernatant and transfer into a microtube.

2- Heat samples at 100°C for 15 mins.

3- Prepare HEK-Blue™ Detection by solubilizing the powder with 50 ml HEK-Blue™ water.

4- Add 50 µl of each sample in a well of a 96-well plate.

5- Add 50 µl of each control in a well of a 96-well plate.

6- Prepare HEK-Blue™-2 cell suspension using HEK-Blue™ Detection medium.

7- Add 200 µl (~50,000 cells) of cell suspension to each well containing the samples or controls.

8- Incubate the plate at 37°C in a CO_2 incubator overnight (16-24h)

9- Detect the presence of *Mycoplasma* with the naked eye or with a spectrophotometer at 620-655 nm.

PlasmoTest™ Kit Contents:

- HEK-Blue™- 2 cells (3-5 x 10^6 cells)
- HEK-Blue™ Selection (4 x 2 ml) - Antibiotic mix
- Normocin™ (4 x 1 ml)
- HEK-Blue™ Detection (2 x 50 ml)
- HEK-Blue™ water (2 x 60 ml)
- Positive control - Not a living Mycoplasma
- Negative control

Buy the kit once then reorder only the reagents to perform further assays.

Product	Quantity	Code
PlasmoTest™	1 kit (up to 500 samples)	rep-pt
HEK-Blue™ Selection	4 x 2 ml	hb-sel
Normocin™	10 x 1 ml	ant-nr-1
HEK-Blue™ Detection	2 pouches	hb-det
PlasmoTest™ Controls	200 tests	pt-ctr

For more information, download the manual from our website: www.invivogen.com

Most Commonly Used Methods for Detection of Mycoplasma Infection

Methods	Description	Specificity	Ease of use	Duration	Drawbacks
Microbiological Culture	Inoculation of cell culture supernatants on agar, broth, or semisolid media	Virtually all species	+++	~3 weeks	Some species don't grow
DNA Fluorochrome Staining	Staining with DNA specific dye and examination under fluorescent microscope	All species	++	30 mins to several days	False positives
PCR	Amplification of the 16S rRNA mycoplasma gene	8 *Mycoplasma* species	+	3h to 1 day	False positives - Requires specific equipment
Enzymatic Assay	Conversion ADP to ATP catalyzed by mycoplasmal enzymes	All species	+++	20 mins	Expensive - Requires specific equipment

Plasmocin™

The Mycoplasma Removal Agent

Mycoplasma contamination is certainly the cell culturist's worst nightmare. The recommended disposal of contaminated cell cultures can now be avoided by using Plasmocin™, the most efficient mycoplasma removal agent. Plasmocin™ is an innovative antibiotic solution that will allow you to save your valuable cell lines in as little as 2 weeks.

Guaranteed elimination of Mycoplasma from Cell Cultures

Plasmocin™ contains two newly developed bactericidal components strongly active against mycoplasmas and related cell wall-less bacteria. Plasmocin™ can be used to both treat and prevent mycoplasma contamination in cell cultures.

Outstanding Efficiency

Plasmocin™'s potent activity comes from two unique bactericidal components. The first component acts on the protein synthesis machinery by interfering with ribosome translation. The second acts on the DNA replication by interfering with the replication fork. These two specific and separate targets are found only in mycoplasmas and many other bacteria and are completely absent in eukaryotic cells.

No Recurrence of Mycoplasma Contamination

Plasmocin™, in contrast to other anti-mycoplasma compounds, is active both on free mycoplasmas as well as intracellular forms. This advantage is conferred by one component of Plasmocin™ which is actively transported into mammalian cells. It ensures that following treatment with Plasmocin™ a cell culture is not reinfected by mycoplasmas released from intracellular compartments of infected cells.

No Modification of Cellular Metabolism

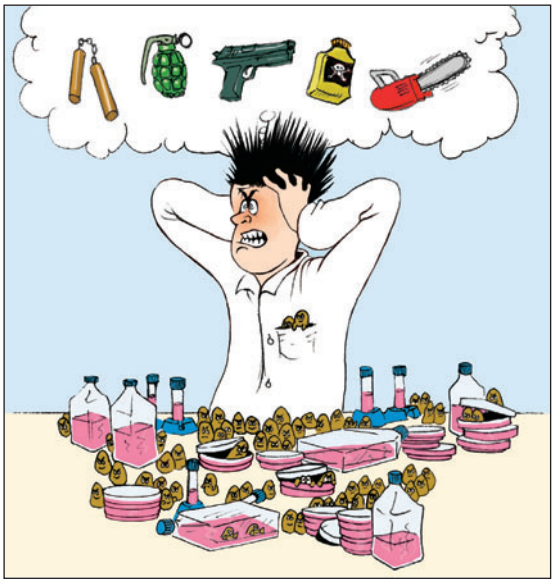
In all animal cell lines tested to date, even at five times the working concentration, no apparent adverse effect on cellular metabolism is observed.

No Acquired Resistance

No resistance in liquid cultures has ever been identified in repeated experiments attempting to measure the mutation rate. Therefore, development of resistant mycoplasma strains is virtually eliminated.

Also Active Against Bacteria Contaminations

Plasmocin™ is also active at low concentrations on a broad range of Gram positive and Gram negative bacteria that are otherwise resistant to the mixture of streptomycin and penicillin, and exhibits no toxicity in eukaryotic cells.



Plasmocin™ Treatment

To eliminate mycoplasmas use Plasmocin™ (ant-mpt) at 25 µg/ml for two weeks in the infected culture.

Plasmocin™ Prophylactic

To prevent mycoplasma contamination, use Plasmocin™ (ant-mpp) at 2.5 - 5 µg/ml on a regular basis in cell culture.

Examples of *Mollicutes* eliminated by Plasmocin™

- *Acholeplasma laidlawii*
- *Mycoplasma arginini*
- *Mycoplasma bovis*
- *Mycoplasma fermentans*
- *Mycoplasma hyorhinis*
- *Mycoplasma orale*

Plasmocin™ is provided as a yellow solution either at a concentration of 25 mg/ml (for treatment) or 2.5 mg/ml (prophylactic). Plasmocin™ is shipped at room temperature. Store at -20°C. Plasmocin™ is stable for at least one year when properly stored.

Product	Quantity	Code
Plasmocin™ (Treatment)	50 mg (2 vials)	ant-mpt
Plasmocin™ (Prophylactic)	25 mg (5 vials)	ant-mpp

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