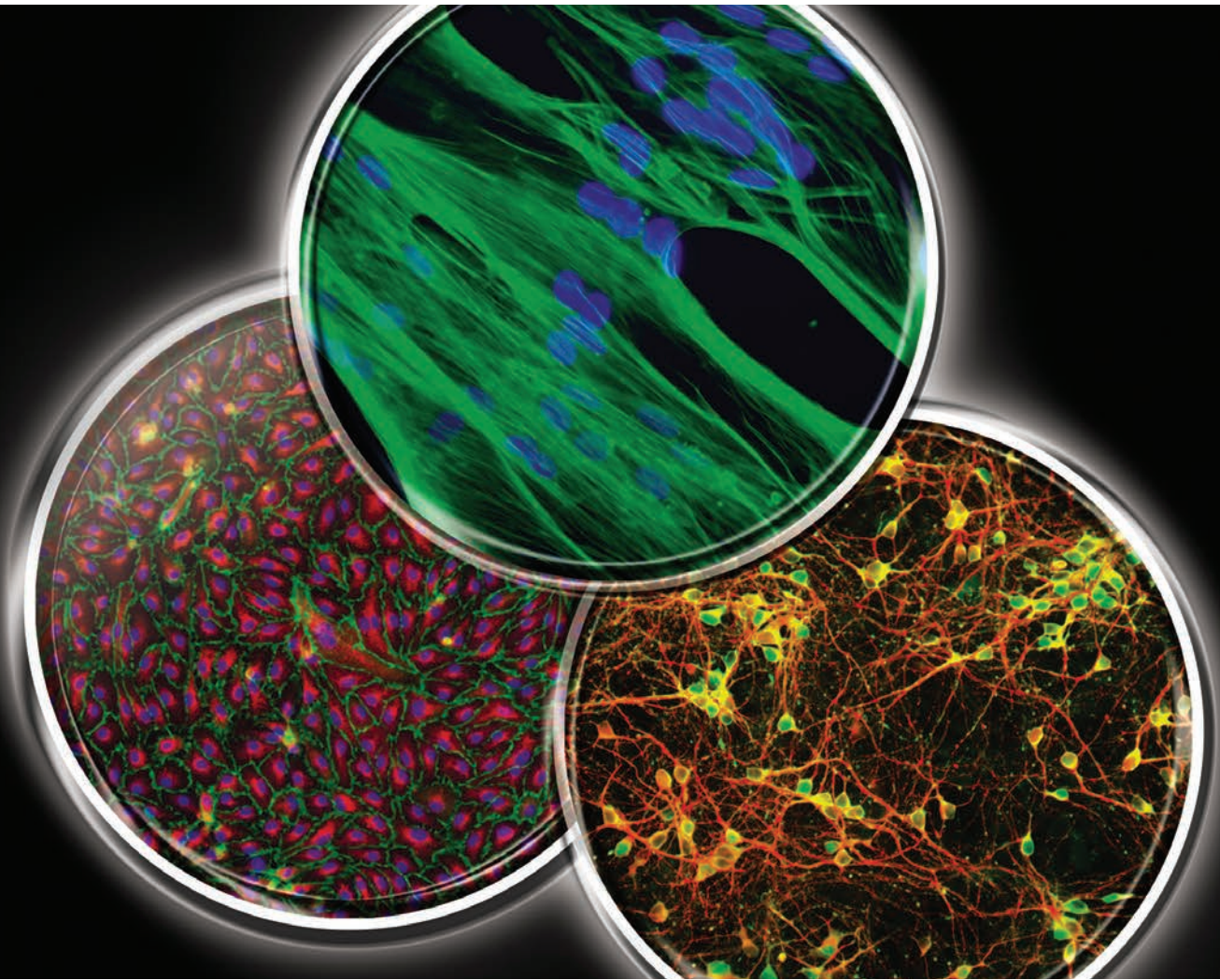


5 Mycoplasma Detection and Prevention



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Mycoplasma Detection and Prevention

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Detection

MycoAlert™ PLUS Mycoplasma Detection Kit 175

Elimination and Prevention

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Introduction

One of the most common contaminants present in cell culture laboratories are mycoplasma. A conservative estimate states that between 15–35% of all continuous cell cultures are contaminated with mycoplasma¹, some estimates are even higher [up to 80 % in some countries]².

What are Mycoplasma?

- Belong to the family Mollicutes including Mycoplasma, Acholeplasma, Ureaplasma, Entomoplasma, Spiroplasma, and other species
- Smallest free-living, self-replicating organisms (size: 0.2 µm–0.8 µm)
- Simple prokaryocytes, lacking a rigid cell wall (surrounded by a single plasma membrane)
- Usually attached to the external surface of the cell membrane
- Relying on their hosts for many nutrients as their biosynthetic capabilities are limited
- Over 180 recognized species
- Six species account for 95% of all mycoplasma infections in cell cultures (*M. orale*, *M. arginini*, *M. fermentans*, *M. salivarium*, *M. hyorhinitis* and *A. laidlawii*)
- Widespread in nature as parasites of humans, mammals, reptiles, insects, and plants

Typical Routes of Mycoplasma Infection in Cell Cultures

- Cross contamination from untested infected cells
- Aerosols created during pipetting
- Using the same bottle of medium for different cell types
- Handling more than 1 cell line in the hood at a time
- Contaminated materials
- Contaminated donor tissue (<1%)
- Direct infection from the researcher

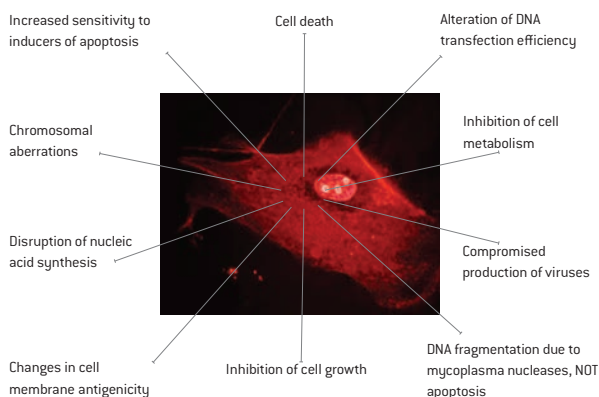
How Insidious Are Mycoplasma?

Contaminations are very difficult to detect or prevent and the presence of mycoplasma can remain undiscovered for months:

- In contrast to bacteria they do not cause visible changes in turbidity or pH
- Not visible under microscopy, even at very high concentrations >10⁷ cfu/mL
- Most routine antibiotics used in cell culture are ineffective against mycoplasma
- They are not routinely removed by filtration

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How Do Mycoplasma Affect Your Cells?



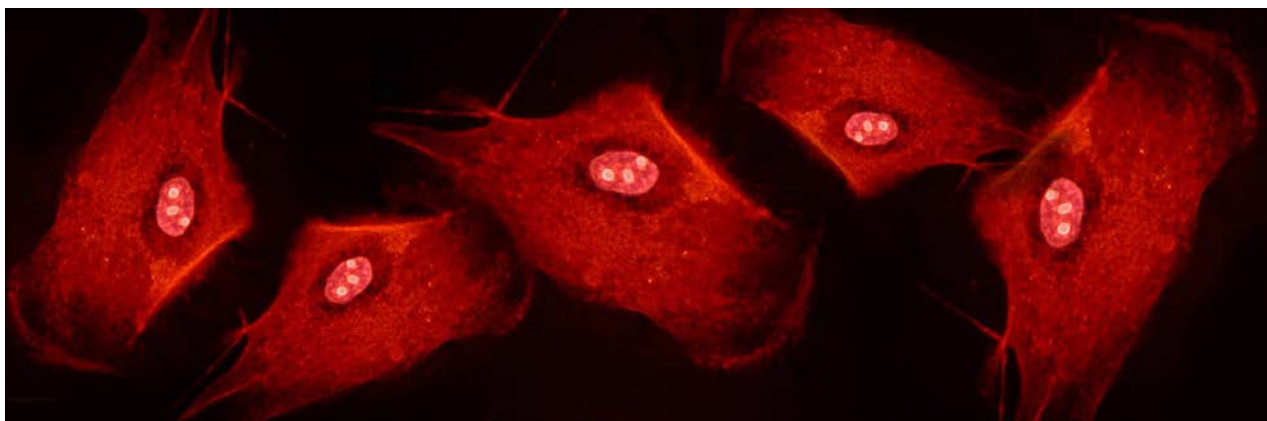
Mycoplasma contamination can seriously impact the reliability, reproducibility, and consistency of experimental results, representing a major problem for basic research as well as for the manufacturing of bioproducts. Standard testing for mycoplasma is an important quality control that should be included in cell culture protocols. We provide a powerful product offering for reliable detection and successful elimination and prevention of mycoplasma contamination:

- **MycoAlert™ Plus Mycoplasma Detection Kit** – Accurate, reliable and universal mycoplasma detection
- **MycoZap™ Mycoplasma Elimination Reagent** – Successful elimination of mycoplasma with low cell toxicity
- **MycoZap™ Prophylactic** – Prevention of mycoplasma contamination in combination with your antibiotic formula of choice
- **MycoZap™ Plus-CL and Plus-PR** – Protection against a broad range of microbial contaminants, such as Gram(+) and Gram(-) bacteria, fungi and mycoplasma

References

1. Drexler H.G., Uphof C.C. (2002): Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. *Cytotechnology* 39: 75–90.
2. Koshimizu K., Kotani H. (1981) in: Procedures for the Isolation and Identification of Human, Animal and Plant Mycoplasmas [Nakamura M., ed.], Saikon, Tokyo, 87-102.

Detection



Introduction 173

Detection
MycoAlert™ PLUS Mycoplasma Detection Kit 175

MycoAlert™ PLUS Mycoplasma Detection Kit

The MycoAlert™ Plus Assay is a selective biochemical test that exploits the activity of mycoplasma cell culture contaminants and the vast majority of 180 mycoplasma species, but are not present in eukaryotic cells. Viable mycoplasma in a test sample are lysed and the enzymes react with the MycoAlert™ PLUS Substrate, catalyzing the conversion of ADP to ATP. By measuring the level of ATP in a sample via a luciferase reaction, both before (read A) and after (read B) the addition of the MycoAlert™ PLUS Substrate, a ratio can be obtained which is indicative of the presence or absence of mycoplasma. The MycoAlert™ PLUS Assay generates a strong light signal providing broad compatibility with plate luminometers and multifunctional readers.

Benefits

- Results in <20 minutes by a simple 2-step assay
- Bioluminescence-based technology
- No DNA extraction necessary
- Convenient enzymatic assay control available for monitoring system performance

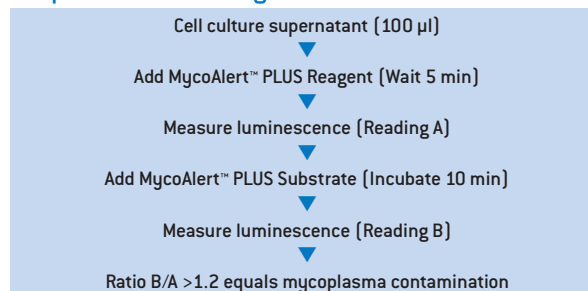
Applications

- Detects all common mycoplasma and acholeplasma contaminations
- Suited for cell culture screening in research environment
- Suited for testing of fresh media, supplements or water

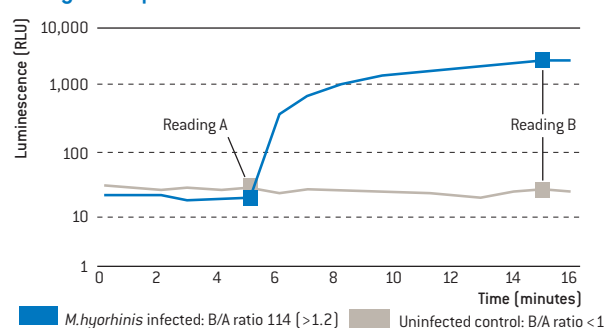
2°C to 8°C; do not freeze prior to reconstitution

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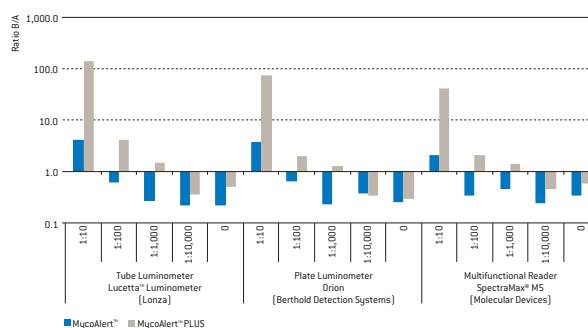
Simple Protocol for Single Tube or 96-well Format



Assay Principle



Kinetics of light emission for uninfected and infected cells. The B/A ratio indicates the presence or absence of mycoplasma.



Comparison of MycoAlert™ and MycoAlert™ PLUS Assay. Dilution series of MycoAlert™ Assay Control demonstrates the increased sensitivity of various luminometers when using MycoAlert™ PLUS Assay.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Size
LT07-701	LT07-701	MycoAlert™ PLUS Mycoplasma Detection Kit	10 reactions
LT07-703	LT07-703	MycoAlert™ PLUS Mycoplasma Detection Kit	30 reactions
LT07-705	LT07-705	MycoAlert™ PLUS Mycoplasma Detection Kit	50 reactions
LT07-710	LT07-710	MycoAlert™ PLUS Mycoplasma Detection Kit	100 reactions
LT27-292	LT27-292	MycoAlert™ PLUS Assay Buffer	20 mL
LT07-518	LT07-518	MycoAlert™ Assay Control Set	10 tests

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Elimination and Prevention



Elimination and Prevention

Myc Zap™ Mycoplasma Elimination Reagent	177
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MycoZap™ Mycoplasma Elimination Reagent

The MycoZap™ Reagent can eliminate detectable mycoplasma contamination in as few as 4 days and has been optimized to clear mycoplasma with minimal toxic effects on the infected cells. It eliminates mycoplasma by using a combination of antibiotic and antimetabolic agents. This approach allows for a highly reliable elimination of mycoplasma that cannot be achieved by the use of antibiotics alone. The MycoZap™ Reagent can be used to eradicate mollicutes, including Mycoplasma, Acholeplasma, Spiroplasma and Entomoplasma species in cell cultures.

■ Benefits

- Efficient mollicute elimination by a combination of antibiotic and antimetabolic agents
- Minimal toxic effects on cells

■ Applications

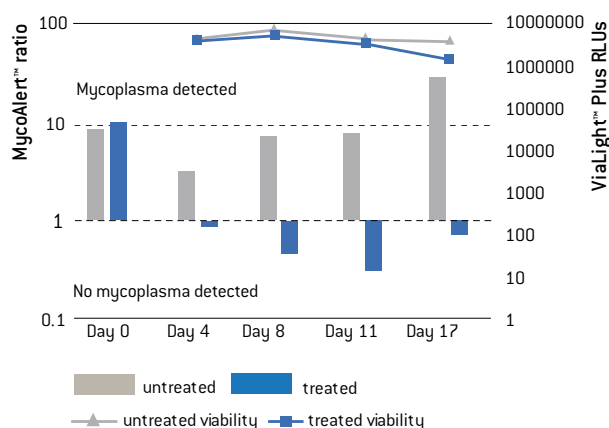
- Eradicates Mycoplasma, Acholeplasma, Spiroplasma, and Entomoplasma
- Suited for a broad range of cell cultures

 2°C to 8°C

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Efficient Mycoplasma Removal with Minimal Effect on Cell Viability



The MycoZap™ Reagent treatment eliminates mycoplasma in as few as 4 days (detected by MycoAlert™ Assay) with minimal impact on cell viability (determined by ViaLight™ Assay).

Ordering Information – Reagent

Cat. No. NA	Cat. No. EU	Product Name	Size
LT07-818	LT07-818	MycoZap™ Mycoplasma Elimination Reagent	1 treatment, for T-25 flask
LT07-918	LT07-918	MycoZap™ Mycoplasma Elimination Reagent	5 treatments, for T-25 flask

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MycoZap™ Antibiotics

MycoZap™ Antibiotics are extremely powerful combinations of innovative antibiotics for the protection of cell cultures from mycoplasma contamination. While MycoZap™ Prophylactic prevents mycoplasma contamination, MycoZap™ Plus offers complete protection against a broad range of common contaminants including mycoplasma.

MycoZap™ Prophylactic

■ Benefits

- Specifically prevents mycoplasma contamination
- Also active against other species of the mycoplasma group like *Acholeplasma* and *Spiroplasma*

■ Applications

- Can be used in combination with other antibiotics (e.g., Pen/Strep) to prevent other microbial contaminants



MycoZap™ Plus-CL and MycoZap™ Plus-PR

■ Benefits

- Active against mycoplasma, Gram(-) and Gram(+) bacteria as well as yeast and fungi
- Complete solution replacing Pen/Strep formulation

■ Applications

- MycoZap™ Plus-CL for protection of cell lines
- MycoZap™ Plus-PR optimized for gentle protection of primary cells

- Immediate use: 2°C to 8°C
- Long-term storage: below -18°C

■ www.lonza.com/mycoplasma

	Mycoplasma Only Solution	Complete Solutions	
	MycoZap™ Prophylactic	MycoZap™ Plus-CL	MycoZap™ Plus-PR
Prevention against mycoplasma	■	■	■
Prevention against	No; but can be used in combination with other antibiotic formula of choice		
– Gram(+) bacteria		■	■
– Gram(-) bacteria		■	■
– Fungi		■	■
– Yeast		■	■
Suited for primary cells	■		■
Suited for cell lines	■	■	

Ordering Information – Reagent

Cat. No. NA	Cat. No. EU	Product Name	Size
VZA-2011	VZA-2011	MycoZap™ Plus-CL Antibiotic	10 × 1 mL
VZA-2012	VZA-2012	MycoZap™ Plus-CL Antibiotic	1 × 20 mL
VZA-2021	VZA-2021	MycoZap™ Plus-PR Antibiotic	10 × 1 mL
VZA-2022	VZA-2022	MycoZap™ Plus-PR Antibiotic	1 × 20 mL
VZA-2031	VZA-2031	MycoZap™ Prophylactic	10 × 1 mL
VZA-2032	VZA-2032	MycoZap™ Prophylactic	1 × 20 mL

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