Lonza

Commonly Asked Questions Concerning MycoAlert™ Products

Q. What kinds of effects could a mycoplasma contamination have on my cells?

A. Mycoplasma contamination can have various effects on eukaryotic cells, including:

– Inhibition of cell metabolism
– Induction of chromosomal or morphological alterations
– Alteration of DNA, RNA, and protein synthesis
– Increased sensitivity to inducers of apoptosis
– Change in membrane antigenicity
– Alteration of transfection efficiency
– Compromising viral production
– Increased cell death

Q. How can I detect mycoplasma contamination of my cells?

A. There are various detection kits available, many of them PCR-based. Our MycoAlert™ Mycoplasma Detection Kit is a simple 20-minute biochemical test that exploits the activity of mycoplasmal enzymes. The presence of these enzymes provides a rapid screening procedure allowing sensitive detection of contaminating mycoplasma in a test sample. Viable mycoplasma are lysed and the enzymes react with the MycoAlert™ Substrate, catalyzing the conversion of ADP to ATP. By measuring the level of ATP in a sample via a luciferase reaction, both before (Reading A) and after the addition of the MycoAlert™ Substrate (Reading B), a ratio can be obtained which is indicative of the presence or absence of mycoplasma. If mycoplasma are not present, the second reading shows no increase over the first. In comparison, reaction of mycoplasmal enzymes with their specific substrates in the MycoAlert™ Substrate leads to elevated ATP levels, resulting in more light output (see Figure 1).

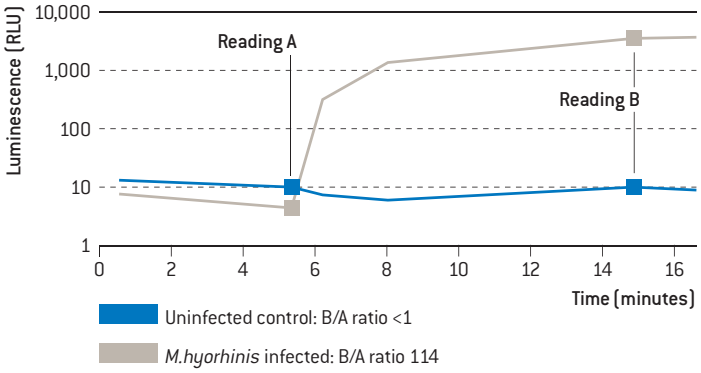

Figure 1
Kinetics of light emission for uninfected and infected cells using the MycoAlert™ Assay. The B/A ratio indicates the presence or absence of mycoplasma.

Q. Can mycoplasma grow in the absence of cells?

A. There is evidence to suggest that mycoplasma can survive in the absence of cells and some may even proliferate, but as a rule, they are much more viable if cells are present. The MycoAlert™ PLUS Kit will detect mycoplasma contamination in cell-free media, sera, and media supplements.

Q. Does the MycoAlert™ Assay recognize the whole spectrum of mycoplasma?

A. The mycoplasma-specific metabolism detected by the MycoAlert™ Assay is present in all members of the mollicute family (Mycoplasma, Acholeplasma, Entomoplasma and Spiroplasma) except Ureaplasma (which are no usual suspects in cell culture). Of all mycoplasma infections of cells grown in culture, 95% are caused by just six species (*M.orale, M.arginii, M.fermentans, M.salivarum, M.hyorhinis* and *A.laidlawii*). The MycoAlert™ Assay can detect these species and many more. To date, we have tested 44 species of Mollicutes including species originally isolated from human, bovine, porcine, ovine, canine, murine, avian, insect, and plant sources. Please contact our Scientific Support Team for further information.

Q. Do bacteria lead to false positive MycoAlert™ Results?

A. No. The lysis reagent in the MycoAlert™ Assay is not strong enough to lyse bacteria or yeast. Also, the enzymes utilized are not very common in bacteria or yeast, but are universal among the mollicute family. Bacterial contamination is characterized by rapid appearance of turbidity in cell culture media. One of the dangers of mycoplasma infections is that they do not produce turbidity, hence the need for a test such as the MycoAlert™ Assay.

Q. Are there positive controls for the MycoAlert™ Assay?

A. The MycoAlert™ Assay Control Set (LT07-518) is available separately and includes a lyophilized positive control (1 mL) and assay buffer (2 mL) for reconstitution. The assay buffer also serves as a negative control. The positive and negative controls are designed for use with the MycoAlert™ Mycoplasma Detection Kit. The assay control set may be used to ensure that the operator and all of the assay reagents are performing properly.
The positive control is proprietary, but does NOT contain mycoplasma. The positive or negative control is simply substituted for a sample in the standard assay protocol. The positive control will give a ratio of >1 while the negative control will give a ratio <1.

Q. What sample volume do I need for accurate results with the MycoAlert™ Assay? Do I need cells or only supernatant?

A. The standard protocol calls for taking 2 mL of culture supernatant, spinning down any cells and removing 100 µl of supernatant. Smaller initial aliquots of supernatant may be taken to start with, if necessary.

Q. What type of sample do I need for the MycoAlert™ Assay? Can it be stored?

A. The MycoAlert™ Assay requires the use of cell-free supernatant only! Cell supernatant must be spun at 1,500 rpm (200 x g) for 5 minutes to remove any remaining cells. Cells present in the sample will increase the background, resulting in loss of sensitivity and possible interference with detection of low-level infections.
For optimal assay performance, supernatant should be tested as soon as possible after collection. Supernatant can be kept at room temperature, or 4°C, for testing same day. Supernatant can be frozen and stored at -80°C for 6 months. For this purpose, freeze supernatant immediately after spinning out cells. Thaw without the aid of artificial heat and equilibrate to room temperature for at least 15 minutes before testing.

Q. Can I use a MycoAlert™ Assay in 96-well format?

A. Yes. The product instructions describe how to use the MycoAlert™ Assay in 96-well format. Actually, the standard protocol and volumes do not differ from the single tube format. Ideally, you would use the 100-test kit format for a complete 96-well plate.

Q. What microtiter plates would you recommend for running a MycoAlert™ Assay on a plate luminometer?

A. We would recommend white-walled, 96-well plates, ideally with an opaque bottom (e.g., Porvair 204003 or Corning Costar CLS3912). These plates give low background and optimal results when used with the MycoAlert™ Assay on a plate luminometer.

Q. Is it possible to quantify mycoplasma with the MycoAlert™ Assay?

A. No. The MycoAlert™ Assay is not quantitative; it has been designed to give a simple "Yes" or "No" answer, pertaining to the presence of mycoplasma.

Q. Is the MycoAlert™ Assay validated for use in a regulatory environment?

A. No. The MycoAlert™ Assay is sold for Research Use Only. For final release testing, we offer the MycoTOOL® PCR Kit.

Q. What is the difference between the MycoAlert™ Assay and the MycoAlert™ PLUS Assay?

A. Both assays are based on the same principle and process. However, the new MycoAlert™ PLUS Assay generates a higher light output and thus is better suited for a broad range of multifunctional readers and for testing of fresh media or supplements.

Q. How can cells be cured of mycoplasma contamination?

A. Where contamination has occurred, and the sample absolutely cannot be discarded, the MycoZap™ Mycoplasma Elimination Reagent has been optimized to eliminate mycoplasma with minimal toxic effects on the cells.
The MycoZap™ Reagent eliminates mycoplasma by using a combination of antibiotic and antimetabolic agents. This approach allows for a highly reliable and definite elimination of mycoplasma that cannot be achieved by the use of antibiotics alone. The MycoZap™ Reagent can be used to eradicate Mollicutes, including all species of mycoplasma, acholeplasma, spiroplasma and entomoplasma in cell cultures. Cultures should be tested with the MycoAlert™ Mycoplasma Detection Kit at regular intervals for 4–6 weeks after mycoplasma elimination to ensure fresh infections do not arise.

www.lonza.com/research
www.lonza.com/mycoalert

Contact Information

North America
Customer Service: +1 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: +1 800 521 0390 (toll free)
scientific.support@lonza.com

Europe
Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com

International
Contact your local Lonza distributor
Customer Service: +1 301 898 7025
Fax: +1 301 845 8291
scientific.support@lonza.com

International Offices
Australia	+ 61 3 9550 0883
Belgium	+ 32 87 321 611
Brazil	+ 55 11 2069 8800
France	0800 91 19 81 (toll free)
Germany	0800 182 52 87 (toll free)
India	+91 22 4342 4000
Japan	+ 81 3 6264 0660
Luxemburg	+32 87 321 611
Singapore	+ 65 6521 4379
The Netherlands	0800 022 4525 (toll free)
United Kingdom	0808 234 97 88 (toll free)

Lonza Walkersville, Inc. – Walkersville, MD 21793