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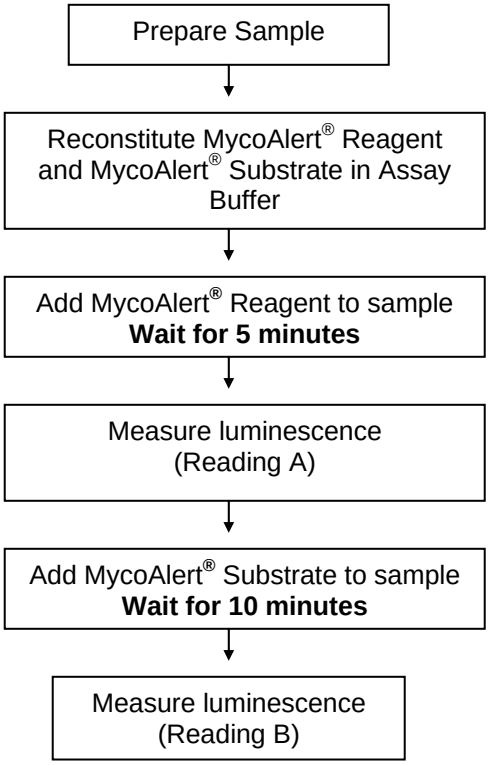
MycoAlert® Mycoplasma Detection Kit

Mycoplasma Detection Assay

Instructions For Use

MycoAlert® Assay Procedure

(For detailed assay procedure see specific protocol pg.3)



Kit Contents

LT07-118 (Sufficient for 10 tests)
1. LT27-217 MycoAlert® Reagent. Lyophilized. 2 x 600 µl vials.
2. LT27-218 MycoAlert® Assay Buffer. 1 x 10 ml bottle.
3. LT27-221 MycoAlert® Substrate. Lyophilized. 2 x 600 µl vials.

LT07-218 (Sufficient for 25 tests)
1. LT27-217 MycoAlert® Reagent. Lyophilized.
5 x 600 µl vials.
2. LT27-218 MycoAlert® Assay Buffer. 1 x 10 ml bottle.
3. LT27-221 MycoAlert® Substrate. Lyophilized
5 x 600 µl vials.

LT07-418 (Sufficient for 50 tests).
1. LT27-237 MycoAlert® Reagent. Lyophilized.
2 x 2.5 ml vials.
2. LT27-218 MycoAlert® Assay Buffer. 1 x 10 ml bottle.
3. LT27-238 MycoAlert® Substrate. Lyophilized.
2 x 2.5 ml vials.

LT07-318 (Sufficient for 100 tests)
1. LT27-216 MycoAlert® Reagent. Lyophilized.
1 x 10 ml vial.
2. LT27-220 MycoAlert® Assay Buffer. 1 x 20 ml bottle.
3. LT27-224 MycoAlert® Substrate. Lyophilized
1 x 10 ml vial.

The kit components should be stored at 2˚C-8˚C. Do not freeze. See kit label for expiry date of the whole kit. See bottle labels for expiry dates of individual components.

Available separately.

LT07-518 MycoAlert® Assay Control Set. (Sufficient for 10 tests)

Intended Use

Mycoplasma are the smallest and simplest prokaryotes. Mycoplasma depend on their hosts for many nutrients due to their limited biosynthetic capabilities. They have long been recognized as common contaminants of cells in continuous culture but their presence may go undetected for months. As the mycoplasma competes with the cells for the nutrients in culture media, one of the first signs is a reduction in the rate of cell proliferation and slight changes in cellular responses including gene expression.
Mycoplasma detection in cell cultures has until now been a long, drawn out process with difficult-to-interpret results. The MycoAlert® Kit is intended for the quick and convenient detection of viable mycoplasma in cell cultures. The speed and convenience of the MycoAlert® Kit allows for the routine testing of cells in culture and commonly used constituents of complete media.
To allow for the early detection of mycoplasma contamination Lonza recommends testing at every cell passage. Frequent testing such as this will indicate when a cell line becomes infected allowing prompt remedial action to be taken.

Principles

The MycoAlert® Assay is a selective biochemical test that exploits the activity of certain mycoplasmal enzymes. The presence of these enzymes provides a rapid screening procedure, allowing sensitive detection of contaminating mycoplasma in a test sample. The 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 both before and after the addition of the MycoAlert® Substrate, a ratio can be obtained which is indicative of the presence or absence of mycoplasma. If these enzymes are not present, the second reading shows no increase over the first, while reaction of mycoplasmal enzymes with their specific substrates in the MycoAlert® Substrate , leads to elevated ATP levels.

This increase in ATP can be detected using the following bioluminescent reaction.

$$\text{ATP} + \text{Luciferin} + O_2 \xrightarrow[\text{Mg 2+}]{\text{Luciferase}} \text{Oxyluciferin} + \text{AMP} + PP_i + CO_2 + \text{LIGHT}$$

The emitted light intensity is linearly related to the ATP concentration and is measured using a luminometer. The assay is conducted at room temperature (18°C-22°C), the optimal temperature for luciferase activity.

Outline of the Method

It is recommended that culture supernatant be centrifuged to remove cells prior to performing the assay.

NOTE: The kit contains all the reagents required to perform the assay.

NOTE: For additional equipment required to perform the assay please see the Equipment section.

1. Spin Cells.
2. 100 µl of culture supernatant is taken as the sample.
3. Add MycoAlert® Reagent.
4. Wait 5 minutes.
5. Read luminescence.
6. Add MycoAlert® Substrate.
7. Wait 10 minutes.
8. Read luminescence.

Reagent Reconstitution and Storage

Please read this section carefully to ensure optimal performance for your assay. Ensure that you follow the correct reagent reconstitution applicable to the kit you have received (10, 25, 50 or 100 tests). After reconstitution, reagents require at least 15 minutes to equilibrate.

The MycoAlert® Reagent and MycoAlert® Substrate are supplied as lyophilized pellets. These are reconstituted in the MycoAlert® Assay Buffer (supplied) to produce the working solutions for use in the assay.

Use reconstituted Reagent and/or Substrate within 5 hours, or 5 days if stored at 2°C-8°C. Unused components can be aliquotted into polypropylene tubes and stored at –20°C for up to six months. Once thawed the reagent and/or substrate must not be refrozen and should be allowed to reach room temperature before use without the aid of artificial heat.

10-and-25 test kits (LT07-118 and LT07-218).

1. Preparation of MycoAlert® Reagent

- Add 600 µl of the MycoAlert® Assay Buffer into a vial containing the lyophilized MycoAlert® Reagent.
- Replace the white screw cap and mix gently.
- Allow the reagent to equilibrate for 15 minutes at room temperature.

2. Preparation of MycoAlert Substrate

- Add 600 µl of the MycoAlert® Assay Buffer into a vial containing the lyophilized MycoAlert® Substrate.
- Replace the green screw cap and mix gently.
- Allow the substrate to equilibrate for 15 minutes at room temperature.

3. MycoAlert Assay Buffer

- This is provided ready for use. Store at 2°C-8°C when not in use.

50 test kit (LT07-418).

1. Preparation of MycoAlert® Reagent.

- Add 2.5 ml of the MycoAlert® Assay Buffer into a vial containing the lyophilized MycoAlert® Reagent.
- Replace the white screw cap and mix gently.
- Allow the reagent to equilibrate for 15 minutes at room temperature.

2. Preparation of MycoAlert® Substrate.

- Add 2.5 ml of the MycoAlert® Assay Buffer into a vial containing the lyophilized MycoAlert® Substrate.
- Replace the green screw cap and mix gently.
- Allow the substrate to equilibrate for 15 minutes at room temperature.

3. MycoAlert® Assay Buffer.

- This is provided ready for use. Store at 2°C-8°C when not in use.

100 test kit (LT07-318).

1. Preparation of MycoAlert® Reagent

- Add 10 ml of the MycoAlert® Assay Buffer into a vial containing the lyophilized MycoAlert® Reagent
- Replace the white screw cap and mix gently.

- Allow the reagent to equilibrate for 15 minutes at room temperature.

2. Preparation of MycoAlert® Substrate
- Add 10 ml of MycoAlert® Assay Buffer into a vial containing the lyophilized MycoAlert® Substrate.
- Replace the green screw cap and mix gently.
- Allow the substrate to equilibrate for 15 minutes at room temperature.

3. MycoAlert® Assay Buffer
- This is provided ready for use. Store at 2°C-8°C when not in use.

Equipment

1. Instrumentation.
The MycoAlert® Kit requires the use of a luminometer. The parameters of the luminometer should be assessed and the conditions below used to produce the correct programming of the machine.

The assay has been designed for use with cuvette/tube and/or plate luminometers.

Cuvette/Tube Luminometers:
• Read time 1 second (integrated)

Plate Luminometers:
• Read time 1 second (integrated)

Beta Counters:
• Mode - out of coincidence or luminescence.
• Read time 1 second (integrated).

2. Additional Equipment and Consumables.
a. 10 ml sterile pipettes
b. Luminometer cuvettes / white walled microplates
c. Micropipettes - 50-200 µl; 200-1000 µl
d. Bench centrifuge

Selection of Protocols

To ensure that the optimal performance of the assay is achieved for your experiment please make certain that you have carefully read the reagent reconstitution and storage procedure.
Please note: samples of the culture medium should be taken before any further processing steps, e.g. trypsinization.

Protocol for cuvette / tube luminometer

1. Bring all reagents up to room temperature before use.
2. Reconstitute the MycoAlert® Reagent and MycoAlert® Substrate in MycoAlert® Assay Buffer. Leave for 15 minutes at room temperature to ensure complete rehydration.
3. Transfer 2 ml of cell culture or culture supernatant into a centrifuge tube and pellet any cells at 1500 rpm (200 x *g*) for 5 minutes.
4. Transfer 100 µl of cleared supernatant into a luminometer cuvette / tube.
5. Program the luminometer to take a 1 second integrated reading (this is usually the default setting on most cuvette luminometers).
6. Add 100 µl of MycoAlert® Reagent to each sample and wait 5 minutes.
7. Place cuvette in luminometer and initiate the program (Reading A).
8. Add 100 µl of MycoAlert® Substrate to each sample and wait 10 minutes.
9. Place cuvette in luminometer and initiate the program (Reading B).
10. Calculate ratio = Reading B/Reading A.

Protocol for 96 well microplate

Please note all reagents should be added manually.
1. Bring all reagents up to room temperature before use.
2. Reconstitute the MycoAlert® Reagent and MycoAlert® Substrate in MycoAlert® Assay Buffer. Leave for 15 minutes at room temperature to ensure complete rehydration.
3. Transfer 2 ml of cell culture or cell culture supernatant into a centrifuge tube and pellet any cells at 1500 rpm (200 x *g*) for 5 minutes.
4. Transfer 100 µl of cleared supernatant into a luminescence compatible plate. White walled plates (e.g. LT27-102) provide best sensitivity with the MycoAlert® Assay.
5. Program the luminometer to take a 1 second integrated reading.
6. Add 100 µl of MycoAlert® Reagent to each sample and wait 5 minutes.
7. Place plate in luminometer and initiate the program (Reading A).
8. Add 100 µl of MycoAlert® Substrate to each sample and wait 10 minutes.
9. Place plate in luminometer and initiate the program (Reading B).
10. Calculate ratio = Reading B/Reading A.

Interpretation of Results
The ratio of Reading B to Reading A is used to determine whether a cell culture is contaminated by mycoplasma.

The speed and convenience offered by the MycoAlert® Kit means that it provides a unique method for screening cultures for the presence of mycoplasma. As such, it is ideally suited to routine testing of cells in culture. Frequent use of the MycoAlert® Assay will indicate when a cell line becomes infected allowing prompt remedial action to be taken. The MycoAlert® Assay can also be extended to incoming cell lines and the commonly used constituents of complete media.

The interpretation of the different ratios obtained, within each experimental situation, may vary according to the cell types and conditions used. However, the test has been designed to give ratios of less than 1 with uninfected cultures. Cells which are infected with mycoplasma will routinely produce ratios greater than 1.

Table 1. Interpretation of MycoAlert® Assay results illustrating examples of healthy and infected cell lines.

Cell Line	MycoAlert® Assay Ratio	Conclusions
Infected cells		
K562	123.26	Positive
A549	4.10	Positive
U937	8.26	Positive
HepG2	1.27*	Borderline,quarantine and retest in 24 hours
Healthy cells		
HL60	0.72	Negative
COS-7	0.46	Negative

* see Troubleshooting pg. 4

References

- Denecke,J., Becker,K., Jurgens,H., Reinhold,G., and Wolff,J. (1999) Falsification of Tetrazolium Dye (MTT) Based Cytotoxicity Assay Results due to Mycoplasma Contamination of Cell Cultures. *Anticancer Reseach*, **19**: 1245-1248.

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- Razin,S., Yogeu,D, and Naot,Y: (Dec 1998) Moleculer Biology and Pathogenicity of Mycoplasmas. *Microbiol and Molecular Biology Reviews*, 1094-1156.

- Rowe,J.A., Scragg,I., Kwiatkiwski,D., Ferguson,D., Carucci,D., and Newbold,C. (1998) Implications of mycoplasma contamination in Plasmodium falciparum cultures and methods for its detection and eradication. *Molecular and Biochemical Parasitology,* **92**: 177-180.

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- Yong Zhou, James S. Hagood and Joanne E. Murphy-Ullrich. (2004). Thy-1 Expression Regulates the Ability of Rat Lung Fibroblasts to Activate Transforming Growth Factor-ß in Response to Fibrogenic Stimuli *American Journal of Pathology,* **165**: 659-669.

Troubleshooting

High background levels?
Take great care when handling any of the reagents. Skin has high levels of ATP on its surface that can contaminate the reagents leading to falsely high readings. Wear latex gloves or equivalent.

Ensuring optimal performance
The optimal working temperature for all reagents is 22°C. If reagents have been refrigerated always allow time for them to reach room temperature before use.

Pipettes
As with all assays involving manual pipetting in order to gain maximal accuracy and to reduce variability pipettes should be calibrated regularly.

Borderline Ratios around 1
The sensitivity of the assay does allow for detection of covert contamination, and if the ratio is marginally above 1 (for example up to 1.3) it is recommended that the sample be retested. Any cultures maintained in quarantine can be tested after a further 24-48 hours in culture to see if the ratios have increased.

A ratio of less than 1 is produced by the ongoing consumption of ATP over the time course of the assay. Consistent ratios of around 1 demonstrate that this consumption and subsequent drop in RLUs is not being seen and indicates an instrument sensitivity issue.

To try to overcome this, increase the integration time from 1 second up to a max of 10 seconds; check to make sure that filters (not even plain glass) are not present between the sample and detector, and ensure the instrument is in luminescence or "out of coincidence" mode.

Negative RLUs or Ratios
If automatic background subtraction is enabled on the instrument it will cause negative RLUs for the B reading and consequently negative ratios. This option MUST be disabled for the instrument to work correctly with the MycoAlert® Assay.

If technical support is required please contact biotechserv@Lonza.com

When used according to the preceding protocol Lonza's MycoAlert® Assay will provide a sensitive measure of mycoplasma infection in cell cultures. It is intended as a presumptive screening tool, and any positives should be re-tested by a second confirmatory method.

Lonza warrants that this product will perform according to established product specifications. It is sold with the understanding that the purchaser will determine if the product is suitable for his or her application. Lonza shall not be liable for any damages or injury to persons or property arising from the purchase or use of the product.

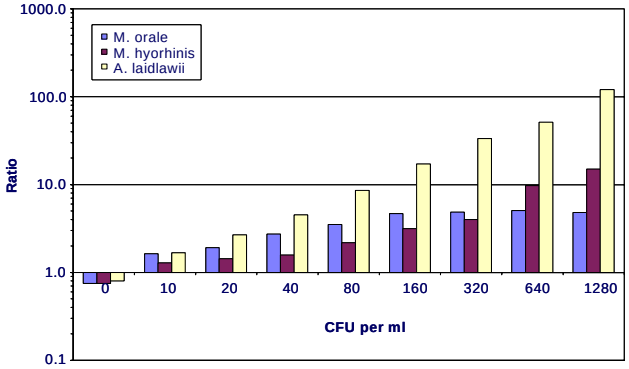

Figure 1: The graph shows a dilution series of M. hyorhinis, *M. orale* and *A. laidlawii* demonstrating the sensitivity of the MycoAlert® Assay Kit.

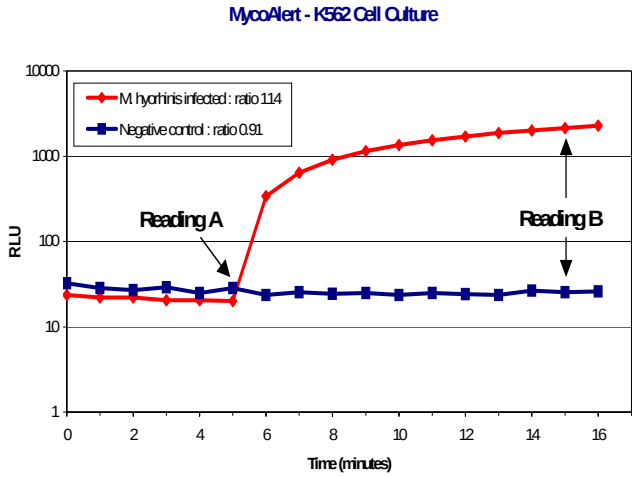

Figure 2: The kinetics of ATP generation in the presence of *M.hyorhinis* contamination.

Ordering Information

LT07-118	MycoAlert® Mycoplasma	10 Test Kit
LT07-218	MycoAlert® Mycoplasma	25 Test Kit
LT07-418	MycoAlert® Mycoplasma	50 Test Kit
LT07-318	MycoAlert® Mycoplasma	100 Test Kit
LT27-102	White Walled Clear Bottom 96 well TC plates–25	
LT07-518	MycoAlert® Assay Control Set	10 Tests

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The MycoAlert Assay is a mycoplasma detection assay. The assay and kits are protected by a granted patent in the UK (GB 2,357,336 B) and by pending patent applications in Canada (CA 2,390,144), Europe (EP 00973075.5), Japan (JP 2001-536755), the UK (GB 0308829.1) and the US (US 10/139,740 and US 60/436,323).

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