

Application Note

DETECTING MYCOPLASMA CONTAMINATION WITH MYCOALERT® ASSAYS AND THE CENTRO LB 963

Abstract

Mycoplasma is a common contaminant in cell cultures which can compromise the reliability of experiments in many laboratories. The MycoAlert® and MycoAlert® PLUS Mycoplasma Detection Kits by Lonza, in combination with the Centro LB 963 Microplate Luminometer, provide a fast, convenient, and reliable way to detect mycoplasma contamination.

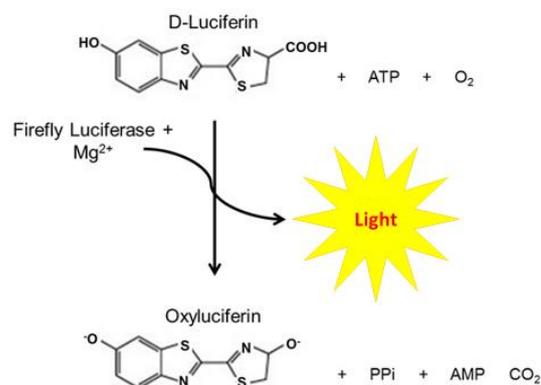
Introduction

Mycoplasma is a genus of bacteria from the class Mollicutes which constitute the simplest and smallest self-replicating and free-living form of life [1]. Several species are pathogenic in humans. Mycoplasma species are a common contaminant in cell cultures: commonly in the range of 15–35% of cultures tested, but it can be as high as 65–80% [2]. Mycoplasma contamination may induce cellular changes, including chromosome aberrations, changes in metabolism, cell growth and gene expression [3]. This means that experiments performed using cells contaminated with

mycoplasma may deliver unreliable results. However, because of their small size (typically 0.1–1 µm), they can't be detected using an optical microscope; this means that other methods have to be used.

The MycoAlert® product line by Lonza includes MycoAlert® and MycoAlert® PLUS, which can identify mycoplasma contamination in cell culture supernatants in just 20 minutes. Both assays use the same assay principle, but MycoAlert® PLUS Assay luminesces much brighter than the original MycoAlert® Assay.

MycoAlert® and MycoAlert® PLUS Assays are based on the activity of certain mycoplasmal enzymes which react with the MycoAlert® or MycoAlert® PLUS Substrates, catalyzing the conversion of ADP to ATP. The ratio between the ATP level before and after the addition of the substrate is indicative of the presence or absence of mycoplasma. The ATP level can be measured using luciferase to oxidize luciferin with the emission of light:



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In this application note we test both MycoAlert® and MycoAlert® PLUS in the Centro LB 963 microplate luminometer.

Centro LB 963 Microplate Luminometer

The Centro is a high-performance, easy to use microplate luminometer for both flash, and glow luminescence applications.

The optimized design provides excellent performance and flexibility:

- Superior sensitivity (<1.8 zmol firefly luciferase)
- Negligible crosstalk (10^{-6})
- Built-in shaker
- JET-injectors
- Temperature control (model-dependent)
- Ergonomic design
- Automation compatibility

The Centro is ideally suited for all luminescent reporter gene assays, immunoassays (LIA, ILMA), cell-based, and biochemical assays.



To meet your compliance requirements, a set of validation tools and optional software providing 21 CFR part 11 compliance are available.

Materials

- Centro LB 963 Microplate Luminometer from Berthold Technologies (Id. Nr. 70325-10).
- MycoAlert® Mycoplasma Detection Kit from Lonza (Catalog # LT07-318).
- MycoAlert® PLUS Mycoplasma Detection Kit from Lonza (Catalog # LT07-710).
- MycoAlert® Control Set from Lonza (Catalog # LT07-518).
- 96-well white microplates from Berthold Technologies (Id. Nr. 23300).
- Pipettes and pipette tips (various volumes).

Methods

Controls and reagents were prepared according to the manufacturer's instructions.

The following dilutions of the MycoAlert® Assay Control were prepared: 1/2, 1/4, 1/8, 1/10, 1/16, 1/100, 1/1 000 and 1/10 000. 100 µL of Assay Control, diluted Assay Control or MycoAlert® Assay Buffer (as negative control) were pipetted in duplicate in the wells of a 96-well white microplate. 2 sets of different dilutions were measured with each kit: for MycoAlert®, undiluted control, 1/2, 1/4, 1/8, 1/16, and negative control; for MycoAlert® PLUS, undiluted control, 1/10, 1/100, 1/1000, 1/10000, and negative control.

To perform the measurement, 100 µL of MycoAlert® Reagent or MycoAlert® PLUS Reagent (depending on the dilution set to be measured) were pipetted manually to each well and the microplate was incubated for 5 minutes at room temperature. After the incubation, luminescence was measured in the Centro luminometer using a counting time of 1 s (Reading A). After the measurement, 100 µL of MycoAlert® Substrate or MycoAlert® PLUS Substrate (depending on the dilution set) were pipetted manually to each well and the microplate was incubated for 10 minutes at room temperature. After the incubation, luminescence was measured again in

the Centro luminometer using a counting time of 1 s (Reading B).

To calculate the results, RLU values of the duplicates were averaged, and the ratio Reading B/Reading A was calculated. Samples with a ratio above 1.2 are considered as positive, samples with a ratio below 0.9 for MycoAlert® (1.0 for MycoAlert® PLUS) are considered negative, and samples in between are considered borderline (quarantine cells and retest after 24 h recommended).

Results

For MycoAlert® the B/A ratio was above 1.2 for all executed dilutions; for MycoAlert® PLUS, a ratio of 2.0 or above was obtained in dilutions up to 1/1 000 (Table 1). As expected, the light intensity of the undiluted control was much higher (31.6 times higher for reading B) when measured using the MycoAlert® PLUS kit than when measured using the MycoAlert® kit, and this resulted in a B/A ratio 16 times higher.

With MycoAlert® the 1/16 dilution of the control was classified as positive, and with MycoAlert®

PLUS even the 1/1000 dilution was classified as positive.

According to the manufacturer's instructions, a luminometer is considered to have enough sensitivity if it produces ratios > 1.0 for dilutions of at least 1/1000 with the MycoAlert® PLUS kit and of at least 1/8 with the MycoAlert® kit. The Centro LB 963 exceeds those requirements, producing ratios of 2.0 and 6.4 respectively, and producing a positive result with the MycoAlert® kit also for the 1/16 dilution.

| MycoAlert® | | | |
|-------------------|-----------------|-----------------|-----------|
| | Reading A (RLU) | Reading B (RLU) | Ratio B/A |
| Undiluted control | 1 494 | 101 931 | 56 |
| 1/2 | 1 836 | 51 952 | 25 |
| 1/4 | 2 088 | 29 011 | 14 |
| 1/8 | 2 280 | 14 505 | 6.4 |
| 1/16 | 2 400 | 7 585 | 3.2 |
| Negative control | 2 598 | 318 | 0.12 |

| MycoAlert® PLUS | | | |
|-------------------|-----------------|-----------------|-----------|
| | Reading A (RLU) | Reading B (RLU) | Ratio B/A |
| Undiluted control | 3 498 | 3 221 724 | 921 |
| 1/10 | 4 608 | 596 777 | 130 |
| 1/100 | 4 800 | 62 151 | 13 |
| 1/1 000 | 4 812 | 9 655 | 2.0 |
| 1/10 000 | 4 980 | 2 574 | 0.52 |
| Negative control | 5 112 | 1 128 | 0.22 |

Table 1. Measurement data and calculated ratios for the MycoAlert® (left) and MycoAlert® PLUS (right) kits. Samples with ratios in red are considered positive and samples with ratios in green are considered negative.

Conclusions

Mycoplasma contamination in cell cultures is widespread in many laboratories [2]. Given the current availability of methods to detect mycoplasma, it is likely that current incidence is declining, but the potential impact of such contamination on cell metabolism and gene expression highlights the importance of routinely testing cell cultures for mycoplasma contamination in any research laboratory.

The MycoAlert® kits by Lonza can identify mycoplasma contamination in cell culture supernatants. In this short study, MycoAlert® PLUS exhibited a higher sensitivity than the standard

MycoAlert®. Thus, the MycoAlert® PLUS kit enables the reliable detection of mycoplasma even with luminometers which are less sensitive than the Centro.

Using the time settings required for MycoAlert® Assays, the Centro can measure a full 96-well plate in less than 2 minutes. The combination of the Centro LB 963 Microplate Luminometer with the MycoAlert® kits provides a fast, reliable, and convenient way to detect mycoplasma contamination in cell cultures.

Acknowledgement

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Not for use in diagnostic procedures.

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