APPLICATION NOTE

MycoAlert Mycoplasma Detection Assays on Molecular Devices Microplate Readers

Introduction

Mycoplasma, the smallest and simplest of the prokaryotes, are common contaminants of cell cultures. Symptoms of mycoplasma contamination include a reduction in the rate of proliferation and changes in cellular responses, including gene expression. Because mycoplasma cannot be detected by simply examining cell cultures under a microscope, a sensitive and reliable assay is needed to determine whether contamination is present. Traditional mycoplasma detection methods involve time-consuming staining or PCR protocols, and the results can be difficult to interpret.

The MycoAlert™ Assay and MycoAlert PLUS Assay from Lonza provide a rapid and convenient way to detect viable mycoplasma in cell cultures using a luminescence microplate reader. The assays take advantage of mycoplasma-specific enzymes found in major mycoplasma species to screen for contamination. The MycoAlert PLUS Assay is the next-generation kit, which provides higher light output than the standard MycoAlert Kit, allowing for the use of a wide range of luminescence readers as well as testing of unused media, media supplements, or water. The setup procedure is the same for both kits.

In the first step of the assay, MycoAlert Reagent is added to cell culture supernatant, lysing viable mycoplasma and initiating a bioluminescent luciferase reaction (Figure 1) that generates light from background ATP. In the second step, MycoAlert Substrate is added and reacts with the released mycoplasmal enzymes to convert ADP to additional ATP. Bioluminescence is then detected on a plate reader to measure the level of ATP present in the sample before and after the addition of the MycoAlert substrate.

Readings taken on a luminescence plate reader before and after substrate addition are used to obtain a ratio that indicates whether mycoplasma is present. If no mycoplasmal enzymes are present, the second reading shows no increase over the first (ratio < 0.9 with the MycoAlert Assay and < 1.0 with MycoAlert PLUS), but if the sample is positive for mycoplasma, the second reading is higher than the first (ratio > 1.2). Figure 2 outlines the assay workflow.

In this application note, we demonstrate how Molecular Devices microplate readers with luminescence detection mode provide superior sensitivity and ease of use for reliable mycoplasma detection using MycoAlert assays. Both the original MycoAlert Assay Kit as well as MycoAlert PLUS, a newer version with increased sensitivity, were tested and shown to be compatible with Molecular Devices microplate readers.

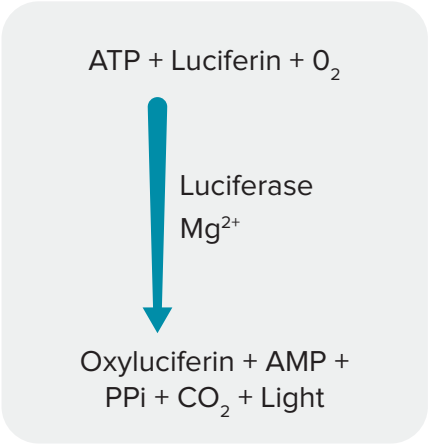

Figure 1: Bioluminescent reaction to detect ATP.

Benefits

- **Sensitive, reliable detection of mycoplasma contamination**
- **Simple add-and-read method for rapid results**
- **Easy interpretation of results**

Materials

- MycoAlert Mycoplasma Detection Kit (Lonza cat. #LT07-318)
- MycoAlert PLUS Mycoplasma Detection Kit (Lonza cat. #LT07-710)
- MycoAlert Assay Control Set (Lonza cat. #LT07-518)
- 96-well polystyrene solid white plates (Greiner cat. #655075)
- Microplate readers
  - » SpectraMax® i3 Multi-Mode Microplate Reader
  - » SpectraMax i3x Multi-Mode Microplate Reader
  - » SpectraMax Paradigm® Multi-Mode Microplate Reader
  - » SpectraMax M5 Multi-Mode Microplate Reader
  - » FilterMax™ F5 Multi-Mode Microplate Reader
  - » SpectraMax L Microplate Luminometer

Methods

Methods for MycoAlert and MycoAlert PLUS Assays are basically the same, except for the concentration range of assay control used, and are detailed below. Each kit was used on the same day that its reagents were reconstituted.

Dilution series of the MycoAlert Positive Assay Control for each assay format were prepared as indicated in Table 1. Dilutions were made in MycoAlert Assay Buffer. 100 µL of each sample was pipetted into a solid, white, flat-bottom 96-well plate. Samples were run in triplicate. MycoAlert Reagent was added to each well and incubated at room temperature for 5 minutes. The plate was then read on Molecular Devices microplate readers with luminescence detection mode (Read A). MycoAlert Substrate was then added to the wells, and the plate was incubated for 10 minutes at room temperature. The plate was then re-read on the plate reader (Read B). Ratios for each sample were calculated as Read B/Read A. Both reads were performed with the instrument settings shown in Table 2. Data were generated and analyzed in SoftMax® Pro Software. Final results were displayed using the bar graph feature available in SoftMax Pro 6.5 or higher.

Results

Sensitivity criteria for luminescence readers have been defined by Lonza for each kit. For the MycoAlert Kit, assay control samples should have a ratio of > 1.2 for dilutions of at least 1:8. For the MycoAlert PLUS Kit, a ratio > 1.2 should be produced by dilutions of at least 1:1000.

All Molecular Devices microplate readers with luminescence detection mode exceeded the sensitivity requirements as specified by Lonza for both MycoAlert and MycoAlert PLUS Kits. Representative data from the SpectraMax i3x Multi-Mode Microplate Reader for both MycoAlert and MycoAlert PLUS assays are shown in Figure 3. Table 3 shows the ratios obtained on all Molecular Devices plate readers tested for the minimum required assay control dilutions for each assay.

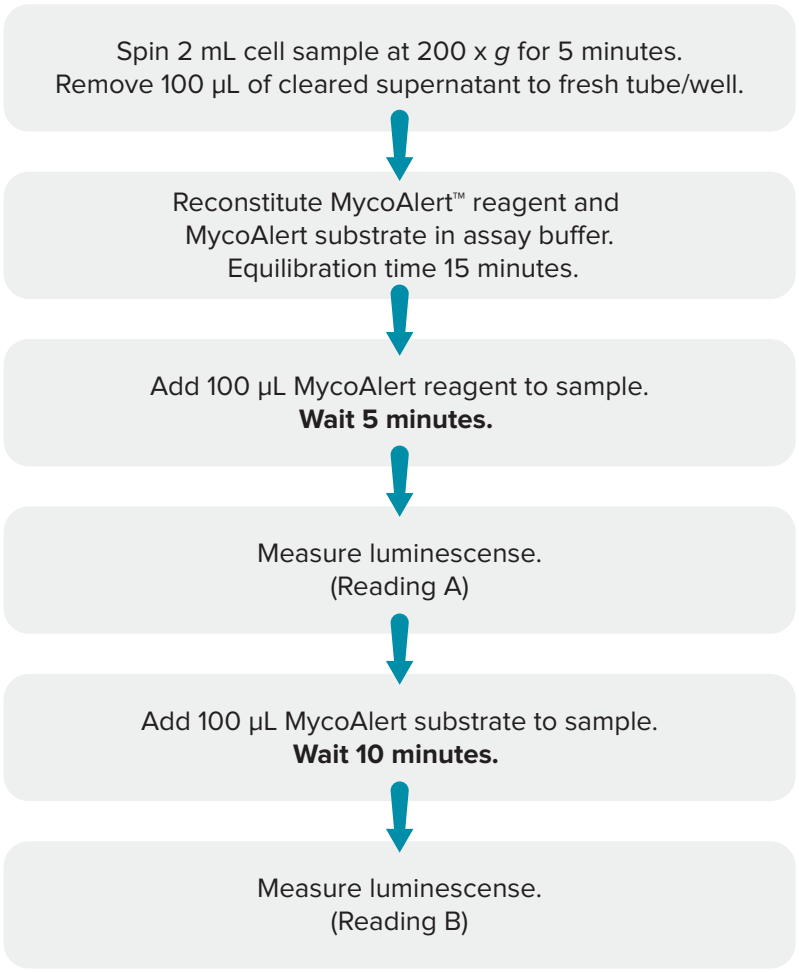

Figure 2. MycoAlert assay workflow. The luminescence measurement for Reading B is divided by Reading A to obtain a ratio indicative of the presence (ratio > 1.2) or absence (ratio < 0.9 with the MycoAlert Assay and < 1.0 with MycoAlert PLUS) of mycoplasma.

MycoAlert	MycoAlert PLUS
Positive control	Positive control
1:2	1:10
1:4	1:100
1:8	1:1000
1:16	1:10000
Negative control	Negative control

Table 1. Preparation of standard dilution series for MycoAlert and MycoAlert PLUS Assays. MycoAlert Positive Assay Control was diluted into MycoAlert Assay Buffer at the ratios indicated.

Parameter	Setting
Read Mode	Luminescence* (All Wavelengths)
Integration Time	1 second
Plate Type	96 Well Greiner

Table 2. General instrument settings for the MycoAlert assays. *The SpectraMax Paradigm reader uses a Luminescence Detection Cartridge to detect signal for this assay. All other readers tested use an integrated luminescence detection mode.

Conclusion

The results presented here confirm that all Molecular Devices microplate readers with luminescence detection mode exceed the sensitivity requirements set by Lonza for detecting mycoplasma with the MycoAlert and MycoAlert PLUS Assays. With both kits, minimal increases in assay signal indicative of mycoplasma contamination can easily be detected.

Molecular Devices microplate readers, in combination with MycoAlert Assays, enable sensitive and rapid detection of mycoplasma, ensuring that contamination is readily detected and saving valuable time in the effort to monitor cell culture contamination.

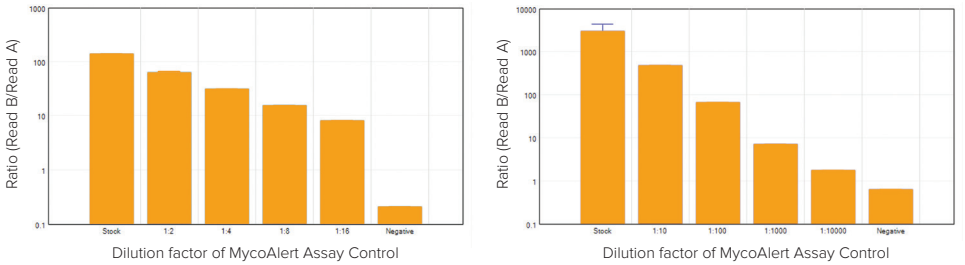

Figure 3: Sensitivity of the SpectraMax i3x reader in detecting increasing dilutions of the MycoAlert Assay Control. Negative samples contained only MycoAlert Assay Buffer. For each kit, the reader was able to detect the lowest dilution as positive. Asterisks indicate the dilution of assay control that should be minimally detectable for each kit. Left: MycoAlert Assay Kit; Right: MycoAlert PLUS Kit.

Microplate Reader	MycoAlert Ratio (1:8 assay control)	MycoAlert PLUS Ratio (1:1000 assay control)
SpectraMax i3x	15.8	7.4
SpectraMax M5	17.8	5.7
SpectraMax Paradigm	7.8	5.2
FilterMax F5	9.8	2.9
SpectraMax L	9.8	9.0

Table 3. Ratios obtained for assay control at minimum required dilutions. All ratios exceeded the required threshold of 1.2.

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