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MycoAlert Mycoplasma Detection Assay (Lonza) on the SpectraMax L Microplate Luminometer

SPECTRAMAX APPLICATION NOTE #21

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INTRODUCTION

Mycoplasma infections are costly to the cell culturist as they change the morphology, viability and metabolism of the contaminated cells. Mycoplasmas are the smallest and simplest prokaryotes and depend entirely on their hosts for many nutrients due to their limited biosynthetic capabilities. Cell culture contamination by mycoplasma is difficult to detect as it does not produce pH changes or turbidity, and culture with antibiotics does little to deter this "invisible" menace.

The MycoAlert Mycoplasma Detection Assay from Lonza Walkersville, Inc. is based on a 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. In the test, viable mycoplasmas are lysed and the released 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. If viable mycoplasma are present they react with their specific substrates in the MycoAlert Substrate and generate ATP. This increase in ATP can be detected using the following bioluminescent reaction:

$$\text{ATP} + \text{Luciferin} + O_2 \xrightarrow[Mg^{2+}]{\text{Luciferase}} \text{Oxyluciferin} + \text{AMP} + \text{PPi} + CO_2 + \text{Light}$$

The emitted light intensity is linearly related to the ATP concentration. Luminescent signal for the MycoAlert Assay was detected using a SpectraMax® L Microplate Luminometer and preconfigured protocol included in the SoftMax® Pro software from Molecular Devices.

METHODS

Sensitivity testing using the MycoAlert assay control

A 100-µL sample of various dilutions of the MycoAlert Assay Control (Lonza) were placed into triplicate wells in a white walled 96-well luminometer plate (Porvair). 100 µL of MycoAlert Reagent (reconstituted in MycoAlert Buffer; Lonza) was added to each well and incubated at room temperature for 5 minutes. The plate was placed into the SpectraMax L Luminometer and a 1-second integrated read taken of each selected well using the MycoAlert protocol in the SoftMax® Pro Software (Read A). 100 µL of MycoAlert Substrate (reconstituted in MycoAlert Buffer) was immediately added to each well and the plate incubated at room temperature for 10 minutes. The plate was placed into the SpectraMax L Luminometer and a 1-second integrated read taken of each selected well (Read B). See Table 1 for instrument settings.

Table 1. Instrument Settings for the SpectraMax L Microplate Luminometer*

Parameter	Setting
Read mode	Lumi Single Wavelength
Integration Time	1 second
Sensitivity	PMT Setting: AutoRange Target Calibration Wavelength: 570 nm
Assay Plate Type	96-well Standard
Dark Adapt	1 minute

* These settings are included in the preconfigured MycoAlert protocol in the SoftMax Pro Software.

The MycoAlert ratio was calculated by dividing Read B by Read A. The results are shown in Figure 1.

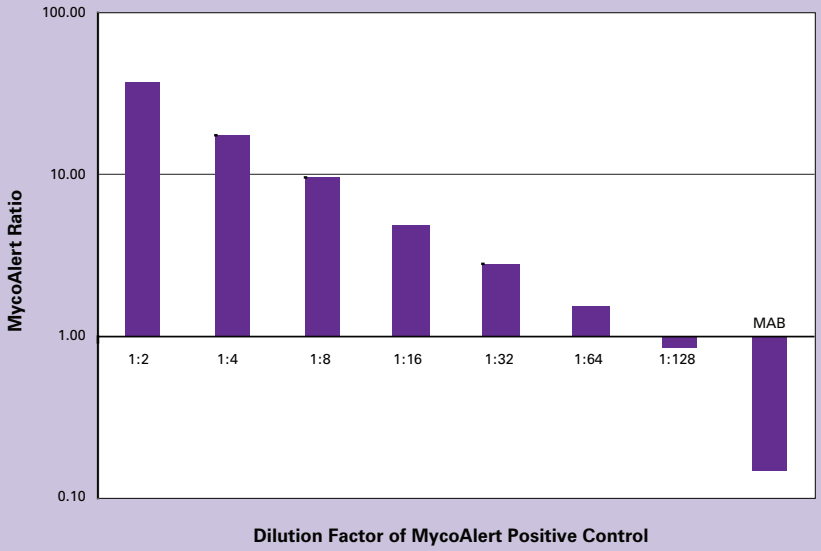
Sensitivity testing using mycoplasma-infected cells
A 2-mL sample of cell culture supernatant was removed from a flask containing K562, human leukemia cells infected with *Mycoplasma hyorhinis.* The cell supernatant was spun at 400 x g for 5 minutes to remove any cells present. (The presence of cells can lead to elevated background levels of ATP and may mask the detection of low level mycoplasma infections).

A 100-µL sample of the cleared culture supernatant was placed into triplicate wells of a white walled 96-well luminometer plate (Porvair). 100 µL of MycoAlert Reagent (reconstituted in MycoAlert Buffer; Lonza) was added to each well and incubated at room temperature for 5 minutes. The plate was placed into the SpectraMax L Luminometer and a 1-second integrated read taken of each selected well (Read A). 100 µL of MycoAlert Substrate (reconstituted in MycoAlert Buffer) was immediately added to each well and the plate incubated at room temperature for 10 minutes. The plate was placed into the SpectraMax L Luminometer and a 1-second integrated read taken of each selected well (Read B).

The MycoAlert ratio was calculated by dividing Read B by Read A. (See Figure 2.) The MycoAlert assay 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. (See Figure 2.)

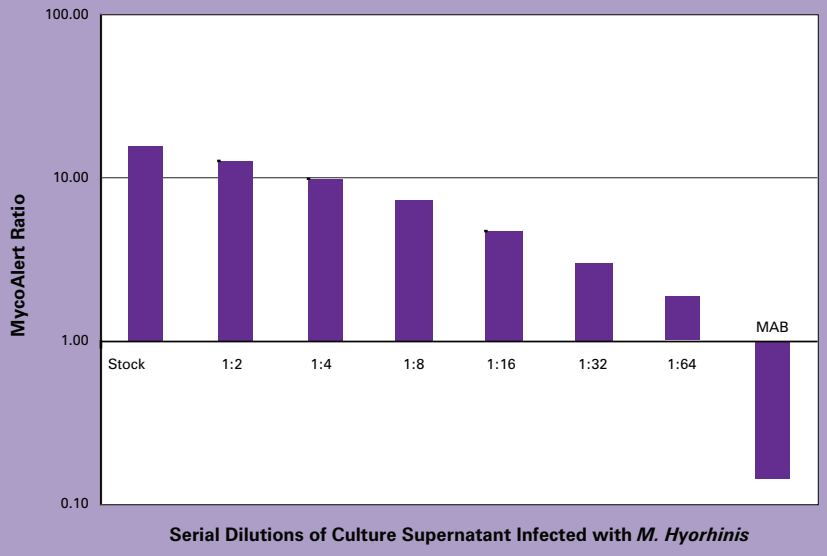
For detailed assay instructions, please visit: http://www.lonza.com/group/en/products_services/products/catalog_new.ParSys.0007.File0.tmp?path=eshop/Instructions-tech_sheets/bioassays/MycoAlert_Mycoplasma_Detection_Kit_18886_.pdf

Sensitivity with the MycoAlert Assay Control (Figure 1)



Sensitivity of the SpectraMax L luminometer in detecting increasing dilutions of the MycoAlert Assay Control. (MAB denotes MycoAlert Assay Buffer.)

Sensitivity with Mycoplasma-Infected Cells (Figure 2)



Sensitivity of the SpectraMax L luminometer in detecting increasing dilutions of mycoplasmal contamination (culture supernatant from mycoplasma-infected cells) using MycoAlert (MAB denotes MycoAlert Assay Buffer).

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RESULTS

Because the MycoAlert assay detects very low levels of ATP in negative samples, there is a requirement for luminometers to detect the low levels of light generated with a high degree of sensitivity. In the guidelines suggested by Lonza, the luminometer should be able to detect a minimum of 1:32 dilution of the MycoAlert Assay Control.

The SpectraMax L Luminometer shows linear detection of the dilution series of MycoAlert Assay control over the suggested 1:32 dilution. The high sensitivity of this luminometer for detection of low levels of light proves its suitability for use with MycoAlert.

The sensitivity testing of this luminometer was investigated further using samples generated from mycoplasma infected cells. Although the definitive number of mycoplasma in this sample was unknown, the combination of MycoAlert Mycoplasma Detection Assay and the SpectraMax L Luminometer provided a large window of detection.

CONCLUSION

The SpectraMax L Microplate Luminometer and SoftMax Pro Software from Molecular Devices combine well with the MycoAlert Mycoplasma Detection Assay from Lonza to provide researchers with a fast and sensitive combination of technologies in the field of mycoplasma detection. High sensitivity and a large window of detection ensure that mycoplasma contamination is readily detected, saving valuable time in the effort to monitor cell culture contamination.

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