



Detecting Mycoplasma using MycoAlert® on the GloMax®-Multi+ Detection System

Simple and fast detection of mycoplasma using the MycoAlert® Mycoplasma Detection Kit and the GloMax®-Multi+ Detection System with Instinct™ Software.

Promega Corporation

Sample Type:

Cell Culture Media

Instrument requirements:

GloMax®-Multi+ Detection System with Instinct™ Software (Cat.# E8032)

MycoAlert® Mycoplasma Detection Kit:

Lonza-50 tests (Lonza Cat. # LT07-418) Protocol (Lonza Cat.# INST-LT07-518)

Additional Materials Required:

Nuclease-Free Water (Cat.# P1193) Vortex mixer Centrifuge

Introduction

Cultured cells are a widely used model system for a variety of reporter and biochemical assays, and the propagation and health of these cells is an important part in setting up and performing a study. There are many types of contamination that can be detrimental to the cell cultures and affect the experiments they are used for. Mycoplasma is one of these contaminants. This simple organism can drastically affect cell viability and growth along with multiple other characteristics and can be very difficult to detect. The MycoAlert® mycoplasma detection kit (Lonza) is a simple mycoplasma detection kit. This Application Note describes how fast and simple it is to use the MycoAlert® mycoplasma detection kit on the GloMax®-Multi+ Detection System with Instinct™ Software. The GloMax®-Multi+ contains luminescence, fluorescence, and absorbance capabilities yet retains the sensitivity of the single-mode instrument. The luminescent module is capable of a dynamic range greater than 8 logs and has a detection limit of 3×10^{-21} moles of luciferase, making it one of the most sensitive instruments available. The microplate format of the reader generates data faster than running multiple single-tube samples. Together these plate reader attributes make the GloMax®-Multi+ an ideal instrument for this assay.

Protocol

1. Bring all reagents to room temperature before use.
2. Reconstitute the MycoAlert® reagent and MycoAlert® substrate in MycoAlert® assay buffer, 2.5ml each. Let rehydrate for 15 minutes at room temperature.
3. Transfer 2ml of cell culture or culture supernatant into a centrifuge tube, and pellet any cells at 1500rpm ($200 \times g$) for 5 minutes.
4. Transfer 100µl of cleared supernatant into wells of 96- or 384-well plate.
5. Add 100µl of MycoAlert® reagent to each sample in the plate, and incubate 5 minutes at room temperature
6. Read luminescence with a 1-second integration using a GloMax®-Multi+ instrument (Reading A).
7. Add 100µl of MycoAlert® substrate to each sample in the plate, and incubate 10 minutes at room temperature.

The GloMax®-Multi+ Detection Systems with Instinct™ Software worked exceptionally well with the MycoAlert® mycoplasma detection kit.

MycoPlasma Detection with GloMax®-Multi+

- Read luminescence with 1-second integration using a GloMax® Multi+ instrument (Reading B)
- Calculate ratio = Reading B/Reading A

Sample Data

Sample data was collected following the protocol outlined for the MycoAlert® assay control set. The control set is designed to test the GloMax®-Multi+ instrument for compatibility with the assay. The test follows the same protocol starting at Step 4 with 100µl of prepared control (Table 1). A luminometer should produce a ratio of <1.0 for the negative control and >1.0 for dilutions of at least 1:8. The sample data provided for the GloMax®-Multi+ were obtained using one half standard reaction volumes, though we recommend using the full reaction volume, according to assay guidelines outlined by Lonza.

Table 1. Control Dilutions. Control dilutions as described in reference 1.

Sample Name	MycoAlert® Positive Control (µl)	MycoAlert® Assay Buffer (µl)
Neat MycoAlert® positive control	100.00	0
1:2 MycoAlert® positive control	50.00	50.00
1:4 MycoAlert® positive control	25.00	75.00
1:8 MycoAlert® positive control	12.50	87.50
1:16 MycoAlert® positive control	6.25	93.75
MycoAlert® negative control	0	100.00

The GloMax®-Multi+ worked exceptionally well and was sensitive enough to pick all dilutions of control (Table 2). The assay also was run at one quarter standard reaction volume, and these reactions passed (data not shown). The sensitivity of the luminescence module allowed the detection of sample even at the 1:16 dilution of the one quarter reaction volume.

Table 2. Results of the GloMax®-Multi+ positive control dilution.

Sample Name	Signal A	Signal B	Signal B/Signal A
Neat MycoAlert® positive control	2189.7	170013	77.72
1:2 MycoAlert® positive control	2739.9	125320	45.74
1:4 MycoAlert® positive control	3524.1	74116	21.03
1:8 MycoAlert® positive control	3511.1	38646	11.01
1:16 MycoAlert® positive control	3759.2	22529	5.99
MycoAlert® negative control	5373.4	524	0.10

Conclusion

GloMax®-Multi+ Detection System with Instinct™ Software is an ideal instrument for the MycoAlert® mycoplasma detection kit.

Reference

- MycoAlert® Assay Control Set Instructions for Use, Lonza Cat.# INST-LT07-518, Lonza web site. Accessed July 9, 2012 (http://bio.lonza.com/uploads/tx_mwaxmarketing-material/Lonza_ManualsProductInstructions_Instructions_-_MycoAlert_Assay_Control_Set_LT07-518.pdf)

Ordering Information

Product	Size	Cat.#
GloMax®-Multi+ Detection System with Instinct™ Software: Base Instrument with Shaking	1 each	E8032
GloMax®-Multi+ Detection System with Instinct™ Software: Base Instrument with Heating and Shaking	1 each	E9032
GloMax®-Multi+ Luminescence Module	1 each	E8041
GloMax®-Multi+ Fluorescence Module	1 each	E8051
GloMax®-Multi+ Visible Absorbance Module	1 each	E8061
GloMax®-Multi+ UV-Visible Absorbance Module	1 each	E9061

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