

Detecting *Mycoplasma* using the MycoAlert™ Kit on the GloMax® 20/20 Luminometer

Promega Corporation



Materials Required

- Cell culture media
- GloMax® 20/20 Luminometer (Cat. #E5311)
- MycoAlert™ Mycoplasma Detection Kit (Lonza Cat.# LT07-418)
- MycoAlert™ Assay Control Set (Lonza Cat.# LT07-518)
- Nuclease-Free Water (Cat.# P1195)
- 1.5ml microcentrifuge tubes
- Vortex mixer
- Microcentrifuge

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *GloMax® 20/20 Luminometer Technical Manual* #TM276 is available at:

www.promega.com/protocols

Overview

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 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 Lonza MycoAlert™ *Mycoplasma* detection kit is a simple *Mycoplasma* detection kit which is based on the measurement of mycoplasmal enzyme activity present in a majority of *Mycoplasma* species. The assay measures ATP generated from a *Mycoplasma*-based luciferase reaction, resulting in a luminescent readout.

This Application Note describes how quick and simple it is to use the MycoAlert™ *Mycoplasma* detection kit on the GloMax® 20/20 Luminometer. The GloMax® 20/20 Luminometer is one of the most sensitive single-tube luminometers on the market today, and offers a linear dynamic range of over 8 logs while also being sensitive enough to detect 1×10^{-21} moles of luciferase. The instrument also is designed to read from the bottom so there are no minimum volume requirements.

MycoAlert™ Assay Protocol

1. Bring all reagents up to room temperature before use.
2. Reconstitute the MycoAlert™ reagent and MycoAlert™ substrate in MycoAlert™ assay buffer, 2.5ml each. Rehydrate for 15 minutes at room temperature.
3. Transfer 2ml of cell culture or culture supernatant into a microcentrifuge tube, and pellet any cells at 1,500 rpm (200 × g) for 5 minutes.
4. Transfer 100µl of cleared supernatant to a 1.5ml microcentrifuge tube.
5. Program the luminometer to take a 1-second integrated reading.
6. Add 100µl of MycoAlert™ reagent to each sample, and wait 5 minutes.
7. Place tube in the GloMax® 20/20 and initiate the program (Reading A).
8. Add 100µl of MycoAlert™ substrate to each sample, and wait 10 minutes.
9. Place tube in the GloMax® 20/20 and initiate the program (Reading B).
10. Calculate ratio = Reading B/Reading A.

Results

Sample data were collected following the protocol outlined for the MycoAlert™ assay control set. The control set is designed to test the GloMax® 20/20 Luminometer 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.

Conclusion

The GloMax® 20/20 luminometer worked exceptionally well and was sensitive enough to also pick up the 1:16 dilution of control (Table 2). The assay was run at one half and one quarter standard reaction volume as well, and these reactions passed (data not shown). The luminescent sensitivity of the GloMax® 20/20 even allowed detection of the 1:16 positive control at one quarter reaction volume.

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Table 1. Control Dilutions. Control dilutions as described for the MycoAlert™ Assay Control Set.

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

Table 2. Results of the GloMax® 20/20 Positive Control Dilution.

Sample Name	Signal A	Signal B	Signal B/Signal A
Neat MycoAlert™ positive control	7,208	489,131	67.86
1:2 MycoAlert™ positive control	8,769	310,011	35.36
1:4 MycoAlert™ positive control	10,931	215,086	19.68
1:8 MycoAlert™ positive control	11,380	105,947	9.31
1:16 MycoAlert™ positive control	10,972	50,811	4.63
MycoAlert™ negative control	14,081	1,674	0.12

