

# *Mycoplasma* Detection using the MycoAlert™ Kit on the GloMax® Discover and GloMax® Navigator Systems

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## Materials Required

- Cell culture
- GloMax® Discover System (Cat.# GM3000)
- GloMax® Navigator System (Cat.# GM2000)
- MycoAlert™ Mycoplasma Detection Kit (Lonza Cat.# LT07-417)
- MycoAlert™ Assay Control Set (Lonza Cat.# LT07-518)
- Nuclease-Free Water (Cat.# P1195)
- White CELLSTAR® cell culture microplate, 96-well, solid bottom (Greiner Bio-One Cat.# 655083)

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

**Protocols:** *GloMax® Discover System Technical Manual #TM397* and *GloMax® Navigator System Technical Manual #TM470* are available at:  
[www.promega.com/protocols](http://www.promega.com/protocols)

*Mycoplasma* can be a major source of cell culture contamination. They function as parasites by exploiting their host's metabolic resources. In cell culture, they can alter cell growth and can affect cellular responses and gene expression. *Mycoplasma* are resistant to commonly used antibiotics and can be undetectable by microscopy. Therefore, a quick, efficient test to detect *Mycoplasma* contamination in cell culture is very useful.

The MycoAlert™ Mycoplasma Detection Kit from Lonza is a quick and convenient detection method of viable *Mycoplasma* in cell cultures. The kit is a luminescent-based assay dependent on the activity of mycoplasmal enzymes. Viable *Mycoplasma* in a test sample (cell supernatant) are lysed and the enzymes react with the MycoAlert™ substrate to produce luminescence (1).

This Application Note describes how quick and simple it is to use the MycoAlert™ Mycoplasma Detection Kit on the GloMax® Discover and GloMax® Navigator plate readers. The GloMax® Instruments are some of the most sensitive luminometers on the market today, with extended dynamic range and minimal well-to-well crosstalk. This allows you to easily measure signals of varying intensity on the same plate.

## MycoAlert™ Assay Protocol

The procedure is performed following the MycoAlert™ Mycoplasma Detection Kit and the MycoAlert™ Assay Control Set Technical Manual (1, 3). The positive control for the MycoAlert™ Mycoplasma Detection Kit, which does NOT contain live *Mycoplasma*, was used to generate a standard curve to assess the linear range of detection on GloMax® plate readers.

1. Reconstitute MycoAlert™ reagent and substrate (provided as lyophilized pellets in the kit (Cat.# LT07-218) by adding 600µl of MycoAlert™ assay buffer in each bottle.
2. Reconstitute MycoAlert™ Assay Control (provided as lyophilized pellets in the kit (Cat. #LT07-518) by adding 1ml of MycoAlert™ Assay Buffer in the bottle.
3. Replace screw caps and gently mix the reconstituted reagents.
4. Allow equilibration for at least 15 minutes at room temperature before use.
5. In a white 96-well plate, prepare a triplicate twofold serial dilution of the MycoAlert™ positive control based on “Testing the Sensitivity of Luminometers” Part B in the *MycoAlert™ Assay Control Set Technical Manual* (3) following Table 1.
6. Collect 2ml of fresh supernatant from the cell culture and centrifuge at 1,500rpm (200 × *g*) for 5 minutes to remove any remaining cells.  
**Note:** For suspension cell culture, supernatant was collected during normal cell passage; for adherent cell lines, supernatant was collected prior to trypsinization.
7. Distribute 100µl of cleared supernatant from test cell cultures prepared in Step 6 into a white 96-well plate in triplicates.
8. Add 100µl MycoAlert™ reagent prepared in Step 1 to samples. Mix by pipetting using a multichannel pipette. Wait for 5 minutes.
9. Measure luminescence on the GloMax® Discover and then on the GloMax® Navigator (1 second integration time) (Reading A).
10. Add 100µl MycoAlert™ substrate prepared in Step 1 to samples. Mix by pipetting using a multichannel pipette. Wait for 10 minutes.
11. Measure luminescence on the GloMax® Discover and then on the GloMax® Navigator (1 second integration time) (Reading B).

12. The ratio between Reading B and Reading A will be the contamination ratio. The negative control should produce a ratio of <0.9 while the MycoAlert™ Assay Control samples with ratios of >1.2 correspond to *Mycoplasma* contamination.

**Table 1. Twofold serial dilutions of the MycoAlert™ positive control.** Prepare three replicates for each concentration. Pipette to mix after each dilution. Remove 100µl from wells after the last 1:64 dilution to keep 100µl volume in all wells.

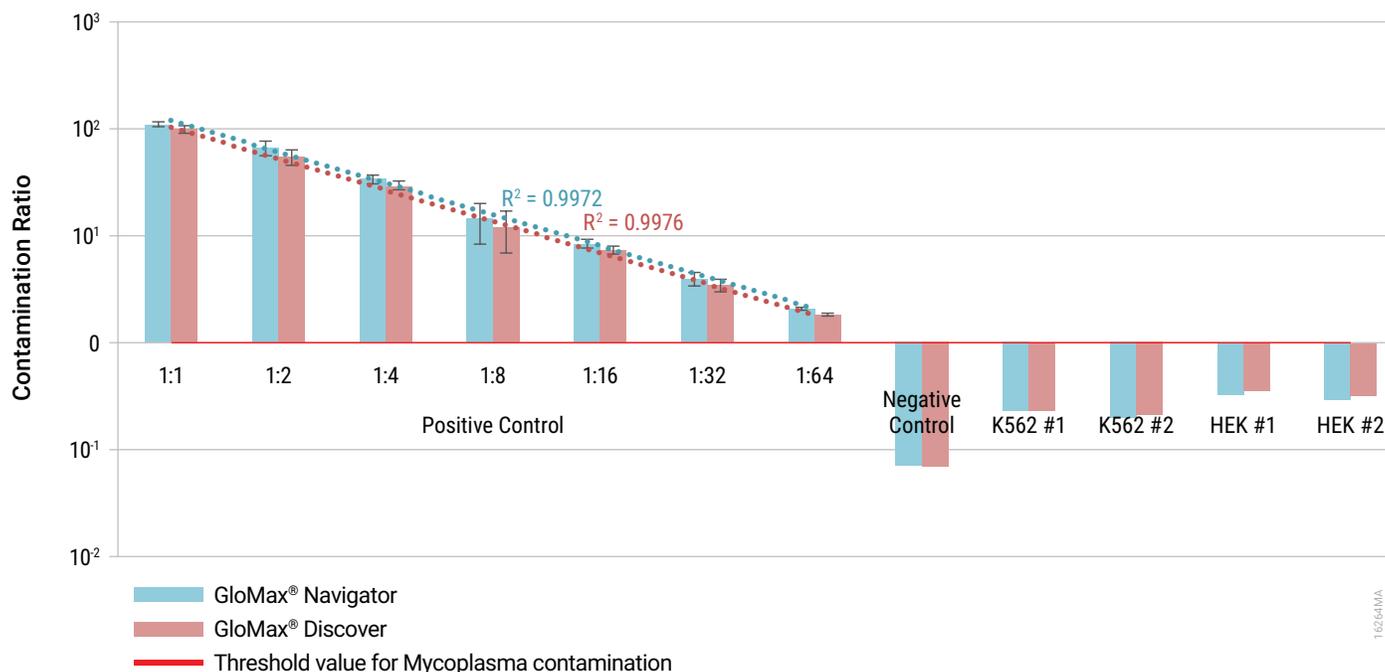
	MycoAlert™ Assay Control	MycoAlert™ Assay Buffer
Neat MycoAlert™ Assay Control	100µl	–
1:2 MycoAlert™ Assay Control	100µl of undiluted MycoAlert™ Assay Control	100µl
1:4 MycoAlert™ Assay Control	100µl of the 1:2 dilution	100µl
1:8 MycoAlert™ Assay Control	100µl of the 1:4 dilution	100µl
1:16 MycoAlert™ Assay Control	100µl of the 1:8 dilution	100µl
1:32 MycoAlert™ Assay Control	100µl of the 1:16 dilution	
1:64 MycoAlert™ Assay Control	100µl of the 1:32 dilution	
MycoAlert™ Negative Control	–	100µl

## Results

We obtained a linear response of luminescence ratio values from the undiluted to the 1:64 dilution of the MycoAlert™ positive control on both GloMax® Discover and GloMax® Navigator instruments (Figure 1). We obtained similar luminescence values on both instruments for all conditions tested. Negative control ratio was 30-fold lower than the ratio value obtained for the 1:64 dilution of the MycoAlert™ positive control. The four different test cell cultures were negative for *Mycoplasma* contamination (Figure 1).

## Conclusions

The GloMax® Discover and GloMax® Navigator plate readers worked exceptionally well to assess *Mycoplasma* contamination in cell cultures using the MycoAlert™ Mycoplasma Detection Kit from Lonza. Excellent sensitivity and R<sup>2</sup> values were observed with both instruments from the linear regression of the standard.



**Figure 1: Contamination ratios for MycoAlert™ positive and negative controls and test cell cultures were obtained using the MycoAlert™ Mycoplasma Detection Kit on the GloMax® Discover and GloMax® Navigator.** Luminescent values were obtained before the addition (Reading A) and after the addition (Reading B) of MycoAlert™ substrates. The ratio between Reading B and Reading A is the contamination ratio. The threshold for *Mycoplasma* detection is indicated by the red line (ratio=1). Linear regression of the ratios for the MycoAlert™ positive control dilutions are shown in dotted lines and the corresponding  $R^2$  are indicated in orange for GloMax® Discover and blue for GloMax® Navigator. The ratios for negative control and all test cell cultures were  $< 1$ , indicating the absence of *Mycoplasma*. Average ratio values are indicated for  $N=3$  replicates  $\pm$  standard deviation; for test cell cultures, average ratio values are indicated for  $N=2$ .

## The GloMax® Discover and Navigator Systems

The GloMax® Discover and Navigator Systems offer superior sensitivity and dynamic range, as well as limited well-to-well crosstalk. The instruments were developed and optimized with Promega cell and gene reporter assays. GloMax® Instruments also allow flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for a wide variety of laboratory applications. The instruments are operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting data to your local network.

## References

1. MycoAlert™ Mycoplasma Detection Kit Technical Manual available from: [https://bioscience.lonza.com/lonza\\_bs/FR/en/download/product/asset/30191](https://bioscience.lonza.com/lonza_bs/FR/en/download/product/asset/30191)
2. MycoAlert™ Assay Control Set Technical Manual available from: [https://bioscience.lonza.com/lonza\\_bs/FR/en/download/product/asset/27672](https://bioscience.lonza.com/lonza_bs/FR/en/download/product/asset/27672)

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