A GloMax® 20/20 Luminometer Method for

Cambrex MycoAlert® Mycoplasma Detection Assay

INTRODUCTION

The GloMax® 20/20 Luminometer in combination with the MycoAlert® Assay from Cambrex Bio Science provides a convenient and rapid method for mycoplasma detection. Mycoplasma are common contaminants of cells grown in culture. With the MycoAlert Assay System, mycoplasma are detected within minutes, making it easy and reliable to use at every cell passage.

The MycoAlert assay follows a two-step format. MycoAlert Reagent is added to the supernatant from cultured cells lysing any mycoplasma present and releasing mycoplasma enzymes. An initial luminescent measurement provides a baseline level of ATP for a particular sample. The addition of MycoAlert Substrate allows the mycoplasma enzymes to catalyze the conversion of ADP to ATP. The final luminescent measurement checks for an increase in ATP due to the presence of these enzymes.

The combination of the GloMax® 20/20 Luminometer with the MycoAlert Assay is capable of detecting very low levels of mycoplasma (Figure 1).

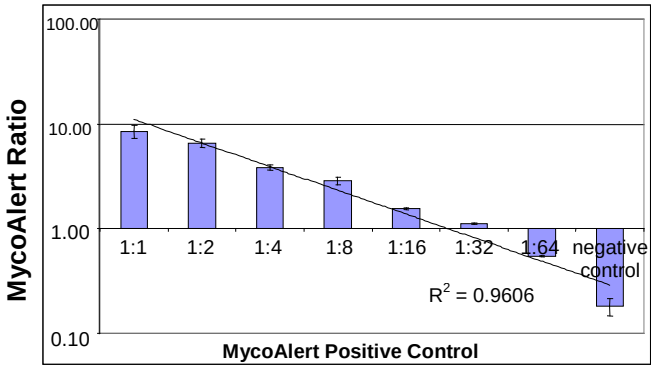


Figure 1. *MycoAlert Assay performed on the GloMax® 20/20 Luminometer using MycoAlert Positive Control.*

MATERIALS REQUIRED

- GloMax® 20/20 Luminometer
- 1.5 mL microcentrifuge tube holder
- Centrifuge
- 1.5 mL microcentrifuge tubes
- Luminometer tubes/cuvettes
- Pipettes (50–200 µL and 200–1000 µL) and the appropriate pipette tips
- MycoAlert Mycoplasma Detection Assay (LT07-118), which contains:
  - LT27-217 MycoAlert Reagent. Lyophilized. 2 x 600 µL vials.
  - LT27-218 MycoAlert Assay Buffer. 1 x 10 ml bottle.
  - LT27-221 MycoAlert Substrate. Lyophilized. 2 x 600 µL vials.

EXPERIMENT PROTOCOL

Note: Wear gloves to prevent ATP contamination from your hands during reagent preparation and sample analysis.

1. Reagent Preparation

- Add 600 µL of MycoAlert Assay Buffer into one vial containing the lyophilized MycoAlert Reagent. Replace white screw cap, and mix gently. Allow the reagent to equilibrate for 15 minutes at room temperature.
- Add 600 µL of MycoAlert Assay Buffer into one vial containing the lyophilized MycoAlert Substrate. Replace the green screw cap, and mix gently. Allow the reagent to equilibrate for 15 minutes at room temperature.

2. Instrument Setup

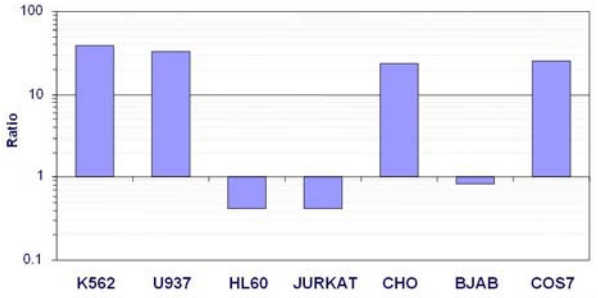
- Turn ON the GloMax® 20/20. A 10-minute warm-up period is recommended but not necessary.
- Choose "Protocols" at the bottom of the touch screen. Select "Default Protocol." The Default Protocol measures samples for 1 second.
- Touch "OK" to go to the Home Screen.

3. Sample Preparation and Analysis

- Transfer 2 mL of cell culture or culture supernatant into a centrifuge tube, and pellet any cells at 1500 rpm (200 x g) for 5 minutes.
- Transfer 100 µL of cleared supernatant into a 1.5 mL clear microcentrifuge tube.
- Add 100 µL of MycoAlert Reagent (white screw cap) to the 1.5 mL clear microcentrifuge tube, and incubate at room temperature for 5 minutes.
- Insert the microcentrifuge tube into the tube holder of the GloMax® 20/20. Close the lid, and touch "Measure Luminescence." Record the result as Reading A.
- Add 100 µL of MycoAlert Substrate to the same microcentrifuge tube, and incubate at room temperature for 10 minutes. Repeat previous step, and record this result as Reading B.
- Calculate ratio = $\frac{\text{Reading B}}{\text{Reading A}}$

RESULTS

- A ratio < 1 indicates an uninfected cell line. Mycoplasma-infected cells routinely produce ratios greater than 1.
- Borderline ratios (e.g., 1.0–1.3) should be retested after 24 hours in quarantine. Any cultures maintained in quarantine may be retested in 24–48 hours to see if the ratios increase.



	K562	U937	HL60	JURKAT	CHO	BJAB	COS7
MycoAlert ™	39	32	0.4	0.4	23	0.8	25
PCR	+	+	-	-	+	-	+

MycoAlert™ compared to Stratagene's Mycoplasma Plus™ PCR kit. Positive cell lines infected with M. hyorhinis

Table 1. *Example of cells analyzed for mycoplasma infection using the MycoAlert system.*

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MycoAlert is a trademark of CBM Intellectual Properties, Inc.

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