

Cambrex Bio Science MycoAlert™ Assay on the LUMIstar plate reader

Anne Cox and Anthony Pitt
Cambrex Bio Science Nottingham, Ltd, UK

Application Note 130

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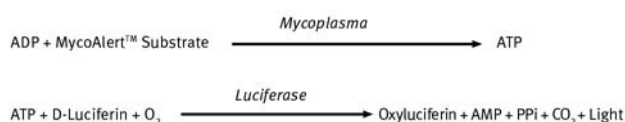


- Detection of mycoplasma in as little as 20 minutes
- Rapid, simple and highly sensitive bioluminescent assay
- Detection down to 10 CFU/mL

Introduction

Mycoplasmas (along with *Acholeplasmas* and other members of the *mollicute* family) are the smallest and simplest prokaryotes. Mycoplasma are a serious contaminant of cell cultures, they are resistant to antibiotics such as penicillin and can alter a wide range of cellular functions while their small size means that even very high levels of contamination can go completely unnoticed. Mycoplasma contaminations of cell cultures have previously been difficult to detect easily and quickly requiring lengthy culturing procedures or subjective staining methods. Cambrex have developed MycoAlert™, a rapid, simple and highly sensitive bioluminescence based assay that allows the detection of mycoplasma in as little as 20 minutes.

By making use of enzymes peculiar to mycoplasma metabolism, the MycoAlert™ Substrate catalyses the formation of ATP from ADP, which can then be detected using the highly sensitive bioluminescent luciferin/luciferase reaction utilised in the MycoAlert™ Reagent as shown in the reaction equation below.



By measuring an increase in ATP over that of background ATP, a ratio can be calculated that when above 1 is indicative of the presence of mycoplasma in the sample (usually cell culture supernatant).

The biochemical activity detected by MycoAlert™ in mycoplasma is conserved across species allowing the assay to detect far more than just the 6 common species (*M. arginini*, *M. salivarium*, *M. fermentans*, *M. hyorhinis*, *M. orale* and *A. laidlawii*) found to routinely contaminate cell cultures. By using the very sensitive ATP detection mechanism of the luciferase/luciferin reaction MycoAlert™ allows very low levels of ATP production to be detected and therefore very low levels of infection. The assay can typically detect 50 CFU per mL or less although specific tests with *M. hyorhinis*, *M. orale* and *A. laidlawii* with independently enumerated samples have shown detection down to 10 CFU per mL.

Any luminometer used with the MycoAlert™ assay has to be sensitive enough to cope with low levels of ATP detection when few or no mycoplasma are present. The BMG LABTECH LUMIstar is a dedicated luminometer that can do this, especially when care has been taken to use correct settings. The purpose of this application note is to show how this is done using the MycoAlert™ Assay Control Set that is supplied by Cambrex.

Materials and Methods

All materials were obtained through normal distribution channels from the manufacturer stated.

- MycoAlert™ Mycoplasma Detection Kit, Cambrex Bio Science, Product code LT07-218
- MycoAlert™ Assay Control Set, Cambrex Bio Science, Product code LT07-518
- LUMIstar Galaxy, BMG LABTECH, UK
- Microplates, white 96-well, Corning Costar, High Wycombe, UK

In addition, consumables such as pipette tips and centrifuge tubes were used as needed from various manufacturers.

All reagents were prepared and the assay run as stated in the MycoAlert™ kit inserts (also on the Cambrex web site, www.cambrex.com). In this instance, manual addition of the reagents rather than by automated injectors was used.

MycoAlert™ Assay Control (a purified enzyme used as a positive control) was diluted serially 1 in 2 with MycoAlert™ Assay Buffer (samples were kept on ice until used). The MycoAlert™ Assay reagent was added to enable the detection of background ATP in the sample and inserted into the LUMIstar to read the luminescence after a 5 minute incubation at ambient temperature (reading A) as shown in figure 1. Once read the cycle was paused and MycoAlert™ Assay Substrate added, the microplate re-inserted into the LUMIstar and the cycle resumed to allow a ten minute incubation at ambient temperature prior to reading the luminescence (reading B).

To ensure the optimum sensitivity, this was repeated with different gain settings for the photomultiplier tube.

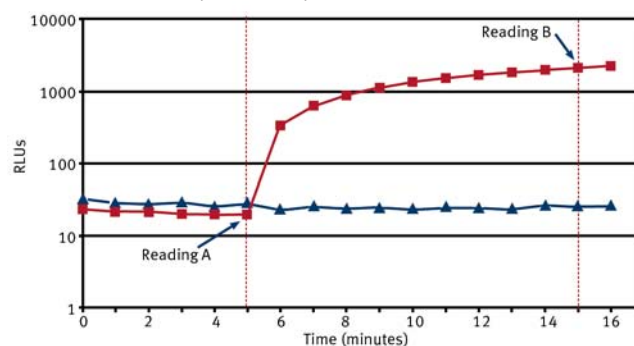


Fig. 1: The kinetic profile of a MycoAlert™ reaction where blue (▲) is a mycoplasma free sample, and red (■) is a mycoplasma positive sample (K562 cell culture infected with *M. hyorhinis*). The profile shows the rapid production of ATP by the mycoplasma positive sample after the addition of the MycoAlert™ Substrate.

LUMIstar parameters used

Read mode: Plate
Gain: HV-Model 2(0-255): various
Optics: Top Optics, 3 mm Lumi Optic
Number of cycles: 2 with pause and 10 minute delay
Integration time: 1 second
Position Delay: Default 0.1 second

After determination of the optimal gain setting to use, samples of *Mycoplasma orale* of known CFU/mL diluted in RPMI were tested.

Results and Discussion

Data was evaluated using Microsoft Excel™ in conjunction with BMG LABTECH LUMIstar Excel™ evaluation package. The MycoAlert ratio was calculated by the final reading B / background reading A, a positive ratio being any value greater than 1. Linear regression performed on the data enabled the most sensitive gain setting to be selected, which would then be used for future tests to enable the presence of mycoplasma to be determined with confidence (table 1 and figure 2).

Table 1: Gain settings for the LUMIstar and resultant R² values.

Gain setting	255	240	125
R ² value	0.9732	0.9901	0.8259

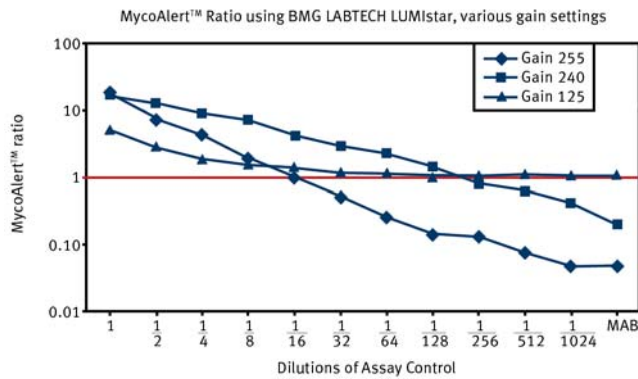


Fig. 2: MycoAlert™ ratio control dilutions using LUMIstar with gain settings of 125, 240 and 255.

Initially the maximum gain setting was selected, but due to low ratios it was decided to reduce this slightly to give increased sensitivity. A further decrease resulted in a positive ratio at all dilutions, but had compromised the linearity, as seen in the lowered R² value, so was not suitable.

The gain setting of 240 was used for *M. orale* dilutions of known CFU as shown in figure 3.

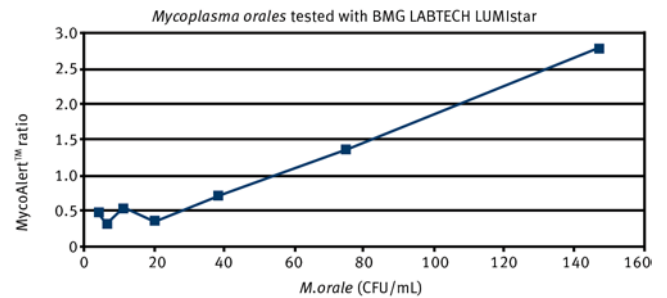


Fig. 3: Testing with gain setting 240, shows 50 CFU/mL can be detected using the BMG LABTECH LUMIstar.

These results were taken from one representative instrument only and should be taken as single measurement data, giving the user an expectation of the sensitive performance of the LUMIstar in conjunction with the MycoAlert™ Kit, but not absolute criteria. Gain settings used may, or may not vary from instrument to instrument.

MycoAlert is a trademark of CBM Intellectual Properties, Inc. MycoAlert Assay covered by patent and U.S. patent pending



Headquarters:

Germany:	BMG LABTECH GmbH	Tel: +49 781 96968-0
Australia:	BMG LABTECH Pty. Ltd.	Tel: +61 3 59734744
China:	BMG LABTECH Co. Ltd.	Tel: +86 10 6424063
France:	BMG LABTECH SARL	Tel: +33 1 48862020
UK:	BMG LABTECH Ltd.	Tel: +44 1296 336650
USA:	BMG LABTECH Inc.	Tel: +1 919 806 1735
	www.bmglabtech.com	info@bmglabtech.com