

Lonza's MycoAlert™ assay on a BMG LABTECH plate reader

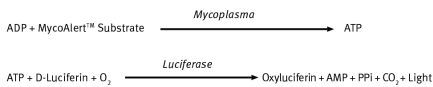
Anne Cox and Anthony Pitt
Cambrex Bio Science belongs now to Lonza

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

Introduction

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. Lonza has developed MycoAlert™, a rapid, simple and highly sensitive bio-luminescence 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. hyorhinitis*, *M. orale* and *A. laidlawii*) found to routinely contaminate cell cultures. 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. hyorhinitis*, *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 microplate reader equipped with luminescence 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 Lonza.

Materials & Methods

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

- MycoAlert™ Mycoplasma Detection Kit, Lonza
- MycoAlert™ Assay Control Set, Lonza
- BMG LABTECH microplate reader
- Microplates, white 96-well, Porvair Sciences

All reagents were prepared and the assay run as stated in the MycoAlert™ kit inserts. 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 measured with the BMG LABTECH plate reader 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, and the cycle resumed to allow a ten minute incubation at ambient temperature prior to reading the luminescence (reading B).

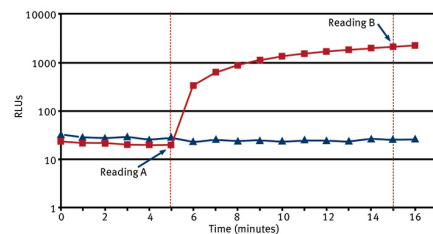


Fig. 1: The kinetic profile of a MycoAlert™ reaction where blue ▲ is a mycoplasma free sample, and red ■ is a mycoplasma positive sample (K562 cell culture infected with *M. hyorhinitis*). The profile shows the rapid production of ATP by the mycoplasma positive sample after the addition of the MycoAlert™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

Instrument settings

Read mode:	Plate
Gain:	3300-3500 depending on reader
Optics:	Top Optics
Number of cycles:	2 with pause and 10 minute delay
Integration time:	1 second
Position Delay:	Default 0.1 second



Results & Discussion

The MycoAlert ratio was calculated by the final reading B/background reading A, a positive ratio being any value greater than 1.

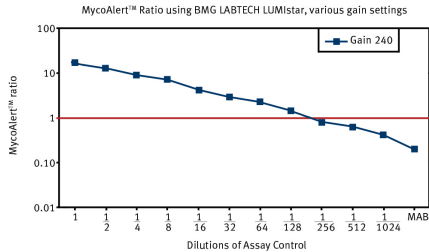


Fig. 2: MycoAlert™ ratio control dilutions.

M. orale dilutions of known CFU are shown in figure 3.

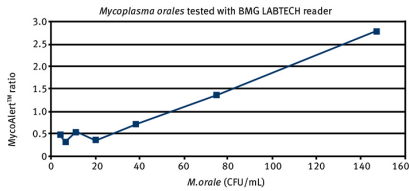


Fig. 3: 50 CFU/mL can be detected using the BMG LABTECH reader.

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 BMG LABTECH readers in conjunction with the MycoAlert™ Kit, but not absolute criteria.

MycoAlert is a trademark of Lonza



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