

Berthold Detection Systems GmbH  
Bleichstrasse 56-68  
D-75173 Pforzheim/Germany

Phone: +49(0)7231/9206-0  
Fax: +49(0)7231/9206-50  
E-Mail: [contact@berthold-ds.com](mailto:contact@berthold-ds.com)  
Internet: [www.berthold-ds.com](http://www.berthold-ds.com)

## Orion II Microplate Luminometer Simplicity 4

### MycoAlert® Mycoplasma Detection Assay (Lonza)

Mycoplasmas are the smallest and simplest prokaryotes which depend on their hosts for many nutrients due to their limited biosynthetic capabilities. Cell culture contamination by mycoplasma is difficult to detect as it does not produce pH changes or turbidity. This “invisible” infection can, however, change the morphology, viability and metabolism of the contaminated cells.

The Lonza *MycoAlert*® assay is a selective biochemical test that exploits the activity of certain mycoplasmal enzymes. The presence of these enzymes provides a rapid screening procedure, allowing sensitive detection of contaminating mycoplasma in a test sample. The viable mycoplasmas are lysed and the enzymes react with the *MycoAlert*® Substrate catalyzing the conversion of ADP to ATP.

By measuring the level of ATP in a sample both before and after the addition of the *MycoAlert*® Substrate, a ratio can be obtained which is indicative of the presence or absence of mycoplasma. If these enzymes are not present, the second reading shows no increase over the first. Reaction of mycoplasmal enzymes with their specific substrates in the *MycoAlert*® Substrate leads to elevated ATP levels.

### Reaction



The emitted light intensity is linearly related to the mycoplasma enzyme concentration.

### Materials

Luminometer: Orion II Microplate Luminometer  
Software: Simplicity 4  
Assay : MycoAlert® Mycoplasma Detection Assay (Lonza)  
Microplates: opaque microplates (solid, white, 96 well), supplied by Porvair

### Methods

**It is strongly recommended that culture supernatant be centrifuged to remove cells prior to performing the assay.**

100µl of culture supernatant was taken as the sample into triplicate wells.  
100µl of MycoAlert® Reagent (reconstituted in MycoAlert® buffer) was added to each well and incubated at room temperature for 5 minutes.

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The plate was placed into the Orion II and a 1s integrated read taken (READ A).

100µl of MycoAlert® Substrate (reconstituted in MycoAlert® Buffer) was added to each well and incubated at room temperature for 10 minutes.

The plate was placed into the Orion II and a 1s integrated read taken (READ B).

The MycoAlert ratio =  $\text{READ B} / \text{READ A}$

The test has been designed to give ratios of less than 1 with uninfected cultures. Cells which are infected with mycoplasma will routinely produce ratios greater than 1.

For detailed assay instructions please visit:

### Example 1

The assay as detailed above was conducted using triplicate serial dilutions of the MycoAlert Control. This procedure is used to test the sensitivity and linearity of luminometers for this assay.

An example graph is shown in Figure 1:

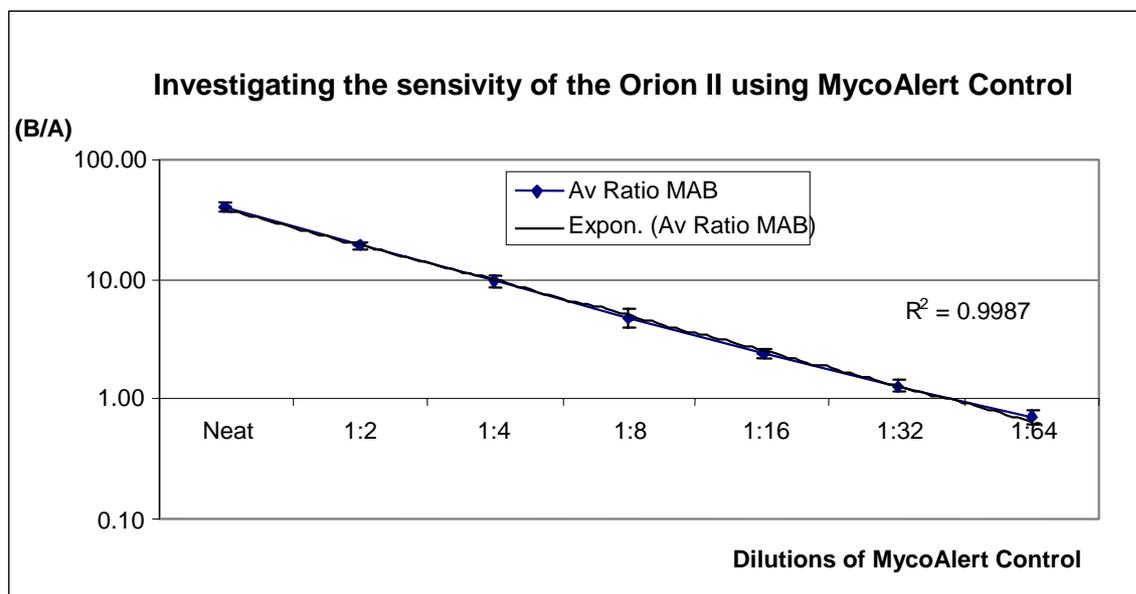


Figure1: Investigating the sensitivity of the Orion II microplate reader for use with MycoAlert Mycoplasma Detection Assay.

### Result 1

The ratio obtained by calculating  $\text{READ B} / \text{READ A}$  was plotted against the relative dilution of MycoAlert positive control. The Orion II microplate luminometer showed excellent sensitivity and linearity in the detection of these standards.

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### Example 2

U937 (human lymphoma) and K562 (human myeloid leukaemia) cells were infected with two strains of bovine mycoplasma (*M. bovis* and *M. bovirhinis*).

The assay as detailed above was conducted using triplicate serial dilutions of the infected cells to look for the presence of mycoplasma.

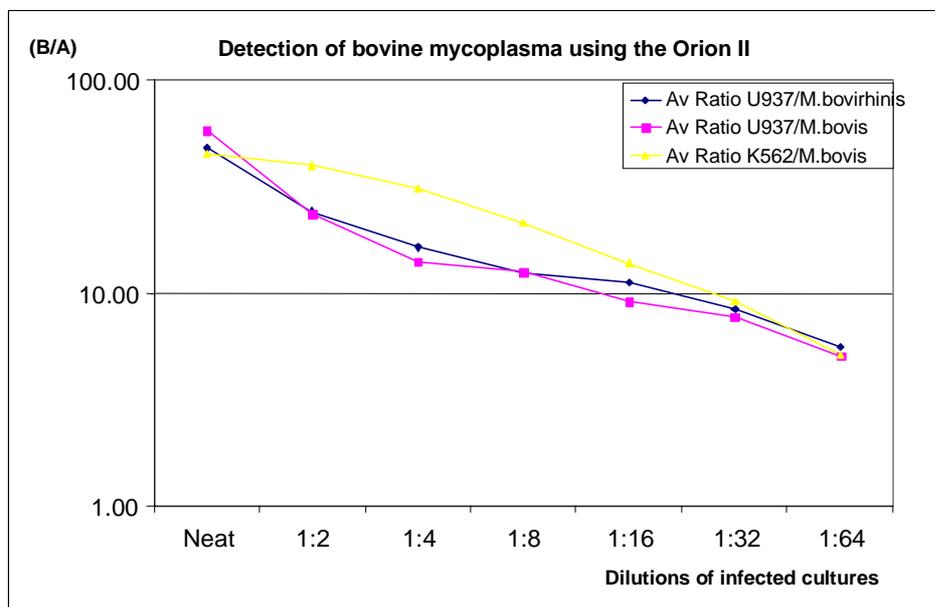


Figure 2: The detection of Bovine mycoplasma using the Orion II. Average  $R^2$  values > 0,98

### Result 2

The ratio obtained by calculating  $READ\ B / READ\ A$  was plotted against the relative dilution of infected cells. The Orion II microplate luminometer again showed excellent sensitivity and linearity in the detection of mycoplasma using MycoAlert<sup>®</sup> Mycoplasma detection Assay

### Acknowledgement

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# Lonza

[www.lonza.com](http://www.lonza.com)