# Mycoplasm detection with the MycoAlert™ assay system

## Tecan Infinite™ F500, Luminescence

## Introduction

### Mycoplasma

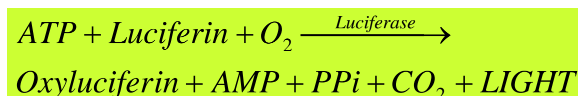
Mycoplasma are among the smallest and simplest prokaryotes, known as parasites in humans, mammals, fish, arthropods etc.. They are indicators for a serious contamination of cell cultures. Because of their small size and their lack of the for bacteria characteristic cell wall, they pass uninterrupted through a typical microbiology filter and they are resistent to antibotics (1,2). A sterile handling of the cell culture is obligatory to avoid contaminations. In the case of an infection by mycoplama a wide range of cellular functions can be affected but do not necessarily lead to a leathal effect. Due to the small size of mycoplasma even high levels of contamination can remain completely unnoticed. Organizations such as the European Collection of Cell Cultures (ECACC) recommend contaminated cells to be destroyed as soon as mycoplasma are discovered (3). Therefore quick and easy detection methods are essential for frequent, routine monitoring of cell cultures. Cambrex BioScience has developed a rapid and highly sensitive bioluminescence method called MycoAlert™ Assay, which allows the detection of all common mycoplasma infections in cell cultures in about 20 minutes (4).

### Assay description

As mycoplasma have unique metabolic processes that derive energy from the infected cell, the MycoAlert assay uses this fact by detecting enzymes that are involved in the mycoplasma metabolism but being absent in the host cell.

The MycoAlert Substrate catalyses the formation of ATP detected by the highly sensitive bioluminescent luciferin - luciferase reaction.

$$ADP + MycoAlert^{TM}\ Substrate \xrightarrow{Mycoplasma} ATP$$



$$ATP + Luciferin + O_2 \xrightarrow{Luciferase} Oxyluciferin + AMP + PPi + CO_2 + LIGHT$$

Performing the assay is notably easy by following the subsequent protocol at room temperature:

Cell Culture supernatant – add MycoAlert reagent (wait 5 min) – measure luminescence (reading A) – add MycoAlert substrate (wait 10 minutes) – measure luminescence (reading B) – calculate ratio B/A

If the calculated ratio is >1 a mycoplasma infection is identified.

For the quantification it is required that the instrument that is used for the MycoAlert assay has to be sensitive enough to detect even very small amounts of ATP. The Infinite™ F500 is proven to perform these sensitive luminescence measurements and the technical note describes how to optimize the signal by using the correct instrument settings.

## Material and methods

### Instrument

Tecan Infinite™ F500 filter-based microplate reader

### Samples / Cell Culture

Different supernatants (S) of a variety of eukaryotic cell cultures were used:

S1 Tecan A3 cells
S2 Tecan H3 cells
S3 CT26 cells
S4 A431 cells
S5 fibroblastoid cells
S6 Wittier cells
S7 skin dendritic cells
S8 Hacat cells
S9 PAA FBS cell culture serum (lot A04304-0318)
S10 sample of incubator water pan

Cell samples were cultivated in their corresponding culture medium and kindly provided by Prof. Barbara Krammer (University of Salzburg) .

The MycoAlert Assay Buffer was used as a negative control (NG) and MycoAlert assay control as a positive control (PC). All samples were measured in triplicates.

### Microplates

96 well LUMITRAC 200 white microplates (Greiner Bio-One)

### Reagents

MycoAlert ™ detection assay (Cambrex BioScience, LT07-318)
MycoAlert Assay Control set (Cambrex BioScience, LT27-236)

All reagents were prepared and the assay was performed according to the Kit Protocol:

1. Transfer 100 µl of culture supernatant, PC and NC,respectively into a well of the microplate

2. Add 100 µl MycoAlert™ Reagent to each sample and wait for 5 minutes

3. Read luminescence at 1 s integration time (Reading A)

4. Add 100 µl MycoAlert™ Substrate to each sample and wait for 10 minutes

5. Read luminescence at 1 s integration time (Reading B)
   Calculate ratio = Reading B / Reading A

### Measurement

Both measurements (reading A and reading B) were performed with the following instrument settings:

| Measurement [A and B] | |
|---|---|
| Plate format | GRE 96 fb, white |
| Mode | Luminescence |
| Attenuation | none |
| Integration time | 1000 msec |
| Settle time | 0 msec |

**Table 1:** Luminescence measurement parameters on Infinite™ F500 using the new Tecan i-control software

### Calculation

The ratio was calculated by dividing the data of measurement B with the data of measurement A. The result was used to determine whether a cell culture is contaminated by mycoplasma (ratio>1) or uninfected (ratio<1).

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## Results and discussion

| | Reading A | | | Reading B | | |
|---|---|---|---|---|---|---|
| **S1** | 794 | 787 | 777 | 216 | 206 | 252 |
| **S2** | 845 | 878 | 856 | 282 | 274 | 289 |
| **S3** | 229 | 241 | 223 | 27833 | 28250 | 27597 |
| **S4** | 482 | 443 | 413 | 187 | 173 | 160 |
| **S5** | 674 | 597 | 618 | 216 | 228 | 240 |
| **S6** | 370 | 296 | 223 | 122 | 126 | 113 |
| **S7** | 810 | 723 | 770 | 329 | 335 | 322 |
| **S8** | 147 | 172 | 140 | 101 | 92 | 107 |
| **S9** | 162 | 178 | 158 | 73 | 95 | 95 |
| **S10** | 642 | 665 | 693 | 85 | 93 | 84 |
| **PC** | 261 | 288 | 253 | 46326 | 45369 | 45759 |
| **NC** | 536 | 521 | 490 | 75 | 88 | 79 |

**Table 2:** MycoAlert Assay – Data Summary.

| | Ratio (B/A) | | |
|---|---|---|---|
| **S1** | 0.3 | 0.3 | 0.3 |
| **S2** | 0.3 | 0.3 | 0.3 |
| **S3** | 122 | 117 | 124 |
| **S4** | 0.4 | 0.4 | 0.4 |
| **S5** | 0.3 | 0.4 | 0.4 |
| **S6** | 0.3 | 0.4 | 0.5 |
| **S7** | 0.4 | 0.5 | 0.4 |
| **S8** | 0.7 | 0.5 | 0.8 |
| **S9** | 0.5 | 0.5 | 0.6 |
| **S10** | 0.1 | 0.1 | 0.1 |
| **PC** | 177 | 158 | 181 |
| **NC** | 0.1 | 0.2 | 0.2 |

**Table 3:** MycoAlert Assay – Ratio (=Reading B/Reading A).

The values obtained from the measurements on the Tecan Infinite™ F500 microplate detection system have clearly confirmed the negative (ratio=0.2) and the positve control (an average ratio of 172). In addition, one cell culture sample out of ten, namely S3 – CT26 cells, was identified as contaminated by mycoplasma.

## Conclusion

This technical note describes the successful performance of the Cambrex BioScience MycoAlert™ Assay on the Tecan Infinite™ F500 filter based microplate reader. According to the achieved data, the performance of the instrument can be described as excellent.

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## Literature

[1] Razin S., Yogev D. and Naot Y., Molecular Biology and Pathogenetic of Mycoplasmas, Microbilogy and Molecular Reviews 1998, 62(4), p.1094-1156

[2] Rottem S. and Barlie M.F., Beware of Mycoplasma, Tibtech, 1993, 11:143-151

[3] ECAAC homepage: http://www.ecacc.org.uk

[4] Cambrex Bio-Science homepage: http://www.cambrex.com

## List of abbreviations

| | |
|---|---|
| ECACC | European Collection of Cell Cultures |
| S | supernatans |
| NG | negative control |
| PG | positive control |

## Acknowledgements

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