Lonza's kinetic kit for endotoxin detection using BMG LABTECH's microplate reader and MARS data analysis

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- **To avoid unwanted inflammatory responses it is important that DNA samples are endotoxin free**
- **The Lonza endotoxin quantitation kit was performed on a filter-based microplate reader**
- **Use of MARS Data Analysis simplifies data interpretation**

Introduction

Endotoxins or lipopolysaccharides (LPS) are undesirable byproducts of gram-negative bacterial preparations which are often found in plasmid DNA and ovalbumin preps. LPS is located in the outer membrane of the bacteria. Even trace amounts can cause a significant inflammatory response. The presence of endotoxin in the blood is called endotoxemia and can lead to sepsis in mammals. Therefore, endotoxins must be detected and eliminated from DNA and protein preparations to avoid unwanted responses in both in vivo and in vitro assays.

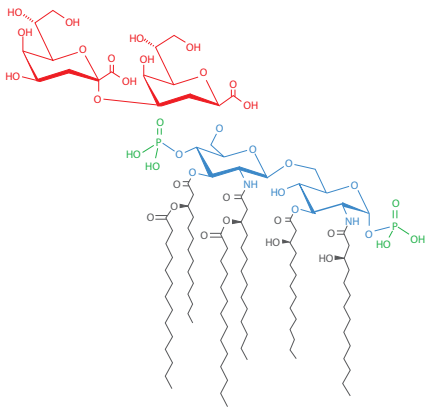
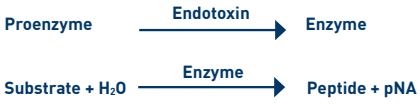


Fig. 1: Structure of the (3-deoxy-D-manno-octulosonic acid)2 Lipid A endotoxin from E. coli K-12. This fi gure is licensed under the Creative Commons Attribution-Share Alike 3.0 Unported licenses and was adapted from: http://www.jlr.org/content/47/5/1097.full.

Lonza has developed a kinetic endotoxin quantitation kit, which utilizes the coagulation properties of horseshoe crab blood in the presence of even low levels of endotoxin. The clottable protein has been isolated and is the active component of the Limulus Amebocyte Lysate (LAL). In the presence of endotoxin, a proenzyme in the LAL is activated which in turn cleaves a colorless peptide, Ac-Ile-Glu-Ala-Arg-pNA, resulting in the release of p-nitroaniline (pNA) which can be detected by continuous absorbance measurements at 405 nm.

Assay Principle



The concentration of the endotoxin is calculated by comparing the reaction times of samples to solutions containing known amounts of endotoxin.

The reaction time is typically defined as the time it takes to produce a 0.2 OD change in absorbance at 405 nm. The endotoxin concentration is inversely proportional to the reaction time so a smaller reaction time indicates a higher endotoxin concentration. The microplate reader offers an easy way of measuring the Kinetic Endotoxin assay and in conjunction with MARS data analysis software can produce reaction times. MARS can also plot the standards and interpolate unknown samples from a linear regression or polynomial fit.

Materials & Methods

- Corning 96 well Microplate, Clear
- Filter-based or spectrometer equipped microplate reader from BMG LABTECH
- Lonza Kinetic-QCL Endotoxin Kit

100 µl of standards and unknowns were measured for absorption at 405 nm in duplicate for a total of 100 min in plate mode (slow kinetics). A reading was taken every 2.5 min for a total of 40 points. Standards included 0.005, 0.05, 0.5 and 5.0 EU/ml, where EU = endotoxic units, a comparative measure of endotoxin activity. Since a baseline correction will be applied, blanks are not required for optimal assay performance.

Instrument settings

Detection Mode:	Absorbance, plate mode kinetic
Optics:	405 nm
No. of cycles:	40
Cycle Time:	150 sec

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Keywords: **Endotoxin, Horseshoe crab, LAL assay, Limulus amebocyte, Lipopolysaccharide**

Results & Discussion

The absorbance measurements over time resulted in different signal curves (Fig. 2).

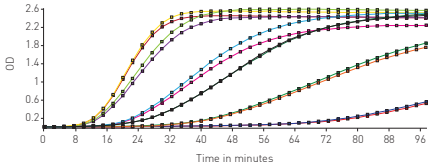

Fig. 2: Signal curves for several endotoxin samples. Figure is directly taken from the MARS data analysis software.

The kinetic data was evaluated utilizing the MARS data analysis software from BMG LABTECH. A baseline correction was applied to the raw data by subtracting cycle 1. The reaction time was calculated by performing a "time to threshold calculation" on the baseline corrected raw data. The threshold value was set for 0.2 OD producing the resulting reaction time in seconds. The average reaction time was plotted against concentration in EU/ml using a linear regression model (Fig. 3).

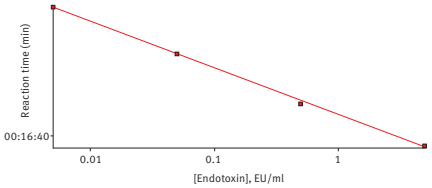

Fig. 3: Linear Regression Fit of Standards (log/log).

The resulting standard curve fit had an R2 value of 0.999 allowing for reliable interpolation of unknown samples (Table 1).

Sample	[Endotoxin], EU/ml calculated from Linear Regression Fit
Unknown 1	4.59
Unknown 2	0.38

Alternatively, the MARS data analysis software facilitated the plotting of standards utilizing a polynomial curve fit. It is recommended that the polynomial order be one less than the number of standards used. In this case a 3rd order polynomial fit could be used.

The MARS data analysis software further allows for the creation of a template for these and other sophisticated calculations (Fig. 4) to provide immediate data reduction and curve fits as soon as the assay is completed.

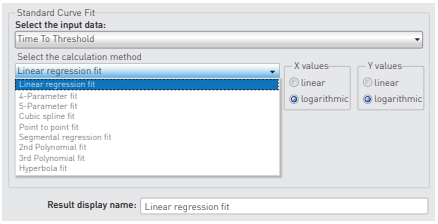

Fig. 4: Overview of standard curve fits in the MARS data analysis software.

Conclusion

The combination of the sensitive microplate reader and the powerful MARS data analysis package allows for easy handling of complex assays such as the Kinetic-QCL Endotoxin kit from Lonza. The MARS data analysis package is standard with all BMG LABTECH readers and can be installed on other computers in the same lab at no additional charge. This assay kit can also be measured by all BMG LABTECH microplate readers that can measure absorbance including the Spectrostar Nano, PHERAstar® *FS*, FLUOstar® Omega, POLARstar® Omega, and CLARIOstar®.