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QUANTIFYING ENDOTOXINS VIA ABSORBANCE OR FLUORESCENCE IN A MULTIMODE PLATE READER.

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Detecting endotoxins is of paramount importance to certify that a product is free of bacterial contamination. Endotoxins are lipopolysaccharides (LPS) that can be found on the outer membrane of certain gram-negative bacteria such as E. coli. The presence of LPS in pharmaceutical solutions can lead to endotoxic shock, inflammation or even sepsis. To certify whether a product is free of endotoxins, several tests can be performed and several kits are available on the market.

The most frequently used test to detect LPS contaminations in a sample is the Limulus amoebocyte lysate (LAL) assay. The LAL assay is based on an extract of amoebocytes from the blood of the Limulus polyphemus, which reacts in the presence of LPS and forms gel clots. The formation of a gel caused by an enzymatic cascade in the lysate indicates the presence of LPS in the sample.

Currently, several variations of this test have been developed to not only assess the presence of LPS but also to quantify it. All kits present on the market still rely on a fundamental enzyme, Factor C (FC), that is part of the LAL assay. The recombinant version of FC, rFC, has been commercialized for over 20 years and is now widely used as it presents several advantages: it does not rely on a live resource that is also an endangered species, it is more consistent, more specific and overall easier to handle.

Factor C (from Limulus blood or recombinant) is activated upon binding to LPS and depending on the kit, its activation leads to the development of a turbid, colored, or fluorescing solution. The intensity of these signals can be measured using plate readers that can read absorbance or fluorescence. Comparison of the signal to that of a standard curve enables quantification of the concentration of LPS in each sample.

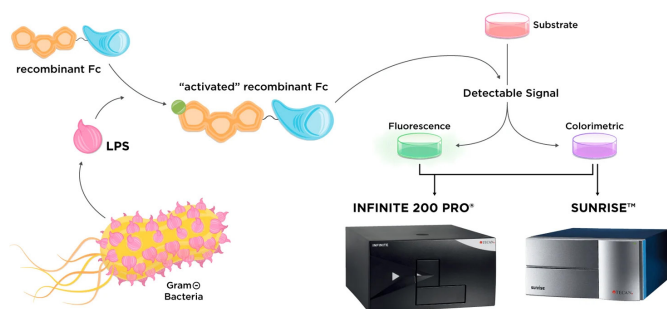


Figure 1: Schematic representation of the functioning rational of an endotoxin detection kit with recombinant Fc

Colorimetric endotoxin detection kits rely on the activation of an enzymatic cascade that starts from Factor C, or a recombinant version of it (rFC) and ends with the cleavage of a reagent that will cause it to become visible and quantifiable. The measurement of this colorimetric signal can be performed via

absorbance reading, using single- or multimode plate readers such as Sunrise™ or Infinite® 200 PRO (Tecan®). Several kits are commercially available to quickly measure and quantify the amount of LPS, such as Endpoint Chromogenic LAL (Lonza), Pyrotell®-T (ACC), or Kinetic-QCL™ (Lonza).

Fluorescence-based endotoxin detection kits work on a very similar concept. A recombinant version of Factor C (rFC) will be activated upon exposure to LPS, leading to the cleavage and release of a fluorophore that can be used to quantify the amount of LPS present in the sample. The fluorescent signal can then be measured with a fluorescence plate reader, such as Infinite 200 PRO. Several kits are commercially available to quantify LPS via fluorescence, such as ENDOZYME® II GO (bioMérieux), ENDOLISA® (bioMérieux), or PyroGene® (Lonza).

TECAN'S SOLUTION FOR YOUR ENDOTOXIN TESTING WORKFLOWS

Infinite 200 PRO and Sunrise are Tecan's suggested products for Endotoxin testing. Thanks to its multimode reading capability and temperature control, Infinite 200 PRO offers the possibility to quantify Endotoxins via colorimetric or fluorescent kits. Sunrise is a robust and accurate single-mode absorbance reader with temperature control, fit for colorimetric endotoxin assays.

Magellan™ is our analysis software and it can be provided together with the necessary methods to analyze and quantify endotoxin assays with the kit of your choice.

Magellan Tracker provides the additional features needed to comply with 21CFR Part 11 for quantification of LPS in a GxP environment.



AUTOMATION OF ENDOTOXIN TESTING

In pharmaceutical applications, endotoxin testing is typically done with large numbers of samples, requiring automation. Tecan liquid handling instrumentation has been successfully used for automating endotoxin tests from major commercial kit providers.

The open nature of the Tecan liquid handling instruments allows for the automation of different kits, even from different providers, on the same instrument. This is ideal in the current trend to change from LAL-based assays to rFC assays. Laboratories can automate their existing assays, required for release of existing products, and new recombinant assays deployed in R&D for development of new products on the same instrument. Also, being able to support multiple kits on Tecan liquid handling

instruments can aid the implementation of robust supply chains for this critical test.

As a concrete example, the ENDOZYME II GO assay from bioMerieux can be automated on the Fluent® platform to process in a single run up to 6 plates, representing up to 120 samples, in under 2 hours. This rFC-based assay using pre-coated plates is very automation-friendly and enables robust automation with high throughput.

REFERENCES

Schwarz et al, Residual Endotoxin Contaminations in Recombinant Proteins Are Sufficient to Activate Human CD1c+ Dendritic Cells, PLoS ONE, 2014, 9(12): e113840

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