

# Quantification of endotoxins from gram-negative bacteria using recombinant factor C assay

*Commonly used acronym: rFC*

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## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Regulatory use - Routine production
<b>Type of method</b>	Other: The test is based on the gene sequence of the horseshoe crab (instead of the blood of horseshoe crab itself)
<b>This method makes use of</b>	Other (e.g. bacteria)

## DESCRIPTION

### Method keywords

LAL

rFC

recombinant factor c  
endotoxins  
pyrogens  
european pharmacopoeia

### **Scientific area keywords**

EDQM  
OCABR  
OMCL  
quality control  
vaccine  
analytical chemistry

### **Method description**

Endotoxins are lipopolysaccharides anchored at the outer membrane of gram-negative bacteria. These pyrogenic compounds can be introduced in pharmaceutical products during their manufacture and could induce severe physiological reactions in humans. This is why endotoxins are dosed, in order to assure quality and safety of products. Testing of endotoxins is well described in the Ph. Eur. (Chapters 2.6.14 & 5.1.10) and several detection methods exist, such as animal-derived *Limulus* amoebocyte lysate (LAL) assays, which are widely used. However, these have limitations such as the use of animals, a high lot to lot variability and interference of complex components such as beta-glucans. Since 2021, assay using recombinant factor C (rFC) is considered as alternative method in the Ph. Eur. (Chapter 2.6.32. ). This method inspired by the LAL assay has the advantages to be animal free and to avoid interference of beta-glucans. It is based on the gene sequence of the horseshoe crab, using a fluorimetric method. This is an end-point detection method (correlation log/log between delta relative fluorescence unit RFU and the concentration of endotoxins). It is performed thanks to a kit bought from a supplier, that includes the

microplate and reagents to perform the tests.

## Lab equipment

fluorimeter

## Method status

Internally validated

Validated by an external party (e.g. OECD, EURL ECVAM,...)

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### Associated documents

## PARTNERS AND COLLABORATIONS

### Organisation

**Name of the organisation** Sciensano

**Department** Risque biologique pour la santé

**Country** Belgium

*Coordinated by*



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