

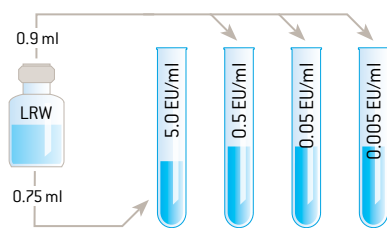
PyroGene™ Recombinant Factor C Assay Procedure Quick Guide

This is a step-by-step guide depicting how to perform the PyroGene™ Recombinant Factor C assay. Prior to initiating the assay procedure, allow reagent vials to equilibrate to room temperature. The incubating microplate reader should also be turned on and a plate template created in the WinKQCL™ Software.

Step 3

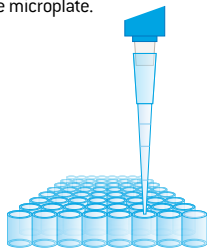
Label the tubes with the appropriate endotoxin concentration and add 0.75 ml of LRW to the 5.0 EU/ml tube. Add 0.9 ml of LRW to each of the remaining tubes.

(Example based on a test with an operating standard curve of 0.005–5.0 EU/ml.)



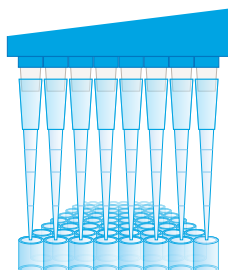
Step 5

Dispense 100 µl of the LRW blank, endotoxin standards, product samples, positive controls, etc. into the appropriate wells of the microplate.



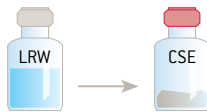
Step 8

Use an eight-channel-pipettor to dispense 100 µl of the working reagent into the appropriate wells of the microplate.



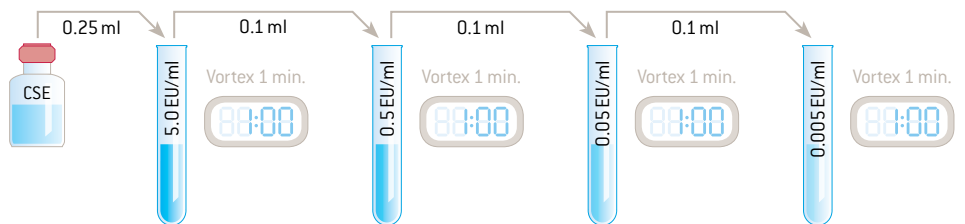
Step 1

Reconstitute Control Standard Endotoxin (CSE) with LAL Reagent Water (LRW) to yield a solution containing 20 EU/ml.



Step 4

Prepare a series of endotoxin standards.



Step 6

Pre-incubate the plate for ≥ 10 minutes at 37°C ± 1°C in the microplate reader.



Step 9

Initiate the test by clicking the OK button in the WinKQCL™ Software.



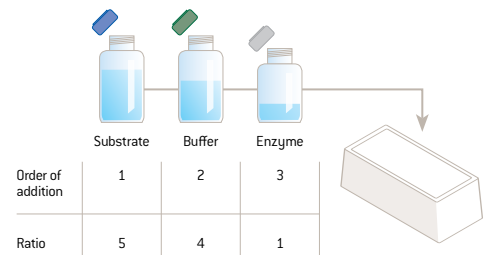
Step 2

Vortex for 15 minutes.



Step 7

During incubation period, prepare the working reagent, which consists of fluorogenic substrate, assay buffer and rFC enzyme solution in a 5:4:1 ratio, respectively. Add enzyme last to the buffered substrate.



Materials, Equipment & Documents Needed

Reagents

- rFC Enzyme Solution
- Fluorogenic Substrate
- rFC Assay Buffer
- Control Standard Endotoxin (CSE)
- LAL Reagent Water (LRW) (# W50-640, W50-100, W50-500)

Please contact your local sales representative for additional information.

Accessories

- Glass dilution tubes (# N207)
- Individually wrapped serological pipettes (optional)
- Tips
- 96-well plates (# 25-340)
- Reagent reservoirs (# 00190035)

Use pyrogen-free accessories that have been qualified for endotoxin testing.

Equipment and Software

- Eight-channel-pipettor
- Incubating fluorescence microplate reader
- WinKQCL™ Software
- Pipettors
- Timer
- Vortex mixer

Supporting Documents

- Certificate of Analysis (CoA), www.lonza.com/coa
- PyroGene™ Recombinant Factor C Endotoxin Detection Assay Package Insert

Points to Consider

- Use matched reagents
- Use glass tubes for making endotoxin dilutions (plastic tubes are not recommended)
- Follow all suggested endotoxin vortexing times
- Use pyrogen-free accessories that have been qualified for endotoxin testing
- Equilibrate reagents to room temperature before use
- Do not vortex the working reagent
- Avoid bubbles when plating reagents into the 96-well plate
- Avoid contaminating samples, standards, LRW and accessories
- Use equipment that has been installed, validated and maintained properly

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