

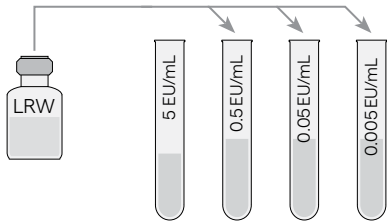
Traditional Kinetic Limulus Amebocyte Lysate (LAL) Assay Procedure Quick Guide

This is a step by step guide depicting how to perform a traditional kinetic LAL 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

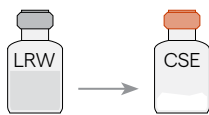
Label the tubes with the appropriate endotoxin concentration and add 0.9 mL of LRW to each.

(Example based on a test with an operating standard curve of 0.005–50 EU/mL.)



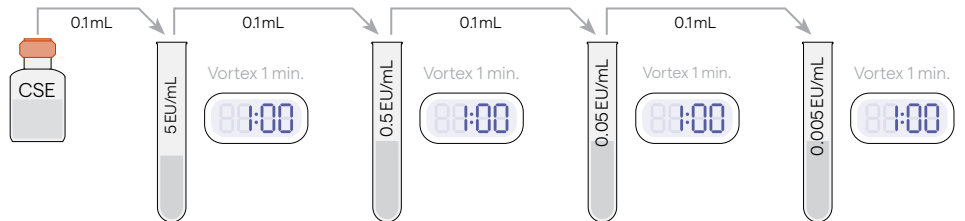
Step 1

Reconstitute Control Standard Endotoxin (CSE) with LAL Reagent Water (LRW) to yield a solution containing 50 EU/mL or 100 EU/mL depending on assay method being used.



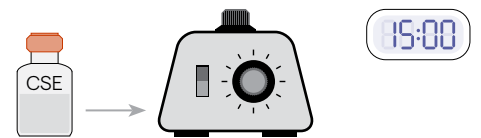
Step 4

Prepare a series of endotoxin standards.



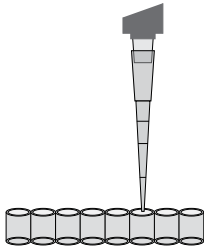
Step 2

Vortex for 15 minutes.



Step 5

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



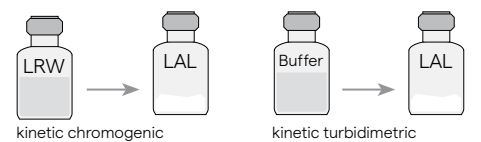
Step 6

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



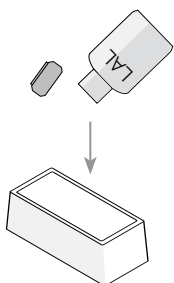
Step 7

Immediately prior to use, reconstitute LAL and gently swirl.



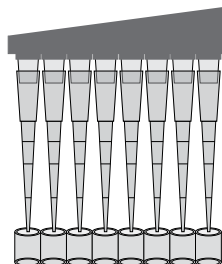
Step 8

Pour LAL into a reagent reservoir and mix gently.



Step 9

Use an eight channel pipettor to dispense 100 µL of LAL into the appropriate wells of the microplate.



Step 10

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



Materials, equipment and documents needed

Reagents

- Limulus Amebocyte Lysate (LAL) Reagent (Kinetic-QCL® or PYROGENT® 5000 Reagent)
 - Control Standard Endotoxin (CSE)
 - LAL Reconstitution Buffer (Required for the PYROGENT® 5000 Kinetic Turbidimetric LAL Assay)
 - LAL Reagent Water (LRW) (# W50-640, W50-100, W50-500)
- Kits are available in a wide range of sizes and configurations. 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 and pipettor
- Incubating absorbance microplate reader
- WinKQCL® Software
- Pipettors
- Timer
- Vortex mixer

Supporting documents

- Certificate of Analysis (CoA), www.lonza.com/coa
- Limulus Amebocyte Lysate (LAL) Kinetic-QCL® Package Insert or Limulus Amebocyte Lysate (LAL) PYROGENT® 5000 Package Insert

Points to consider

- Use matched LAL and CSE reagents
- Plastic tubes are not recommended for making endotoxin dilutions
- 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 LAL
- Avoid bubbles when plating reagents into the 96-well plate
- Avoid contaminating samples, standards, LRW and accessories
- Equipment should be installed, validated and maintained appropriately

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