Pharma&Biotech

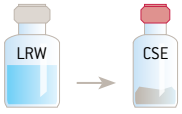
Lonza

Endpoint Chromogenic Limulus Amebocyte Lysate (LAL) Assay Procedure Quick Guide

This is a step-by-step guide depicting how to perform the endpoint chromogenic LAL assay in a 96-well plate. Prior to initiating the assay procedure, allow reagent vials to equilibrate to room temperature. The time and temperature of the assay are critical; therefore, samples, stop reagent and reagents must be ready before starting the incubation.

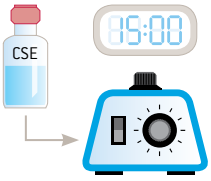
Step 1

Reconstitute Control Standard Endotoxin (CSE) with 1.0 ml of LAL Reagent Water (LRW).



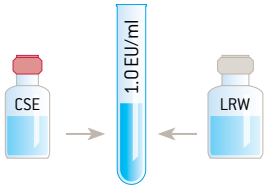
Step 2

Vortex for 15 minutes.



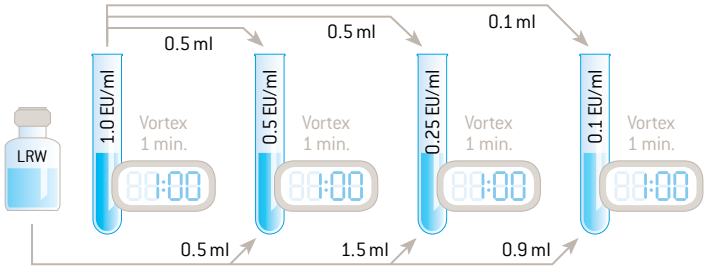
Step 3

Prepare a solution containing 1.0 EU/ml using the endotoxin potency identified on the Certificate of Analysis (CoA).



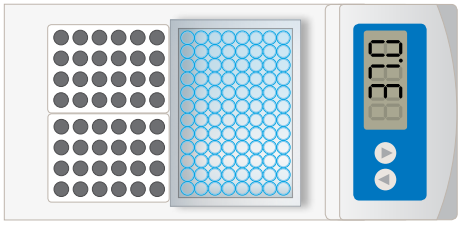
Step 4

Label tubes with the appropriate endotoxin concentration and add LRW to each. Then prepare a series of endotoxin standards. Note: This is not a serial dilution.



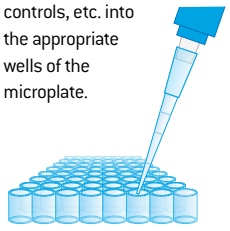
Step 5

Pre-equilibrate the microplate at 37°C ± 1°C in the heating block adapter.



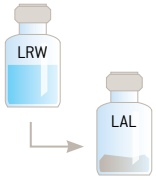
Step 6

Dispense 50 µl of the LRW blank, endotoxin standards, product samples, positive controls, etc. into the appropriate wells of the microplate.



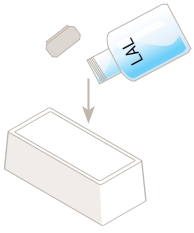
Step 7

Immediately prior to use, reconstitute the LAL and gently swirl.



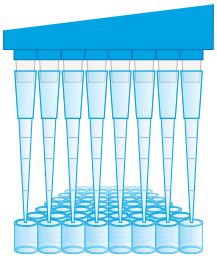
Step 8

Pour LAL into a reagent reservoir and mix gently.



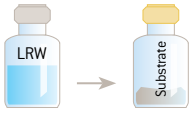
Step 9

At time zero (T=0), use an eight channel pipettor to dispense 50 µl of LAL into the appropriate wells of the microplate. Briefly remove the microplate from the heating block adapter and tap to facilitate mixing. Incubate for 10 minutes.



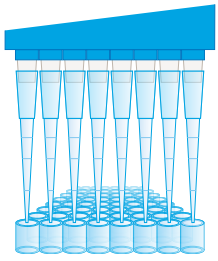
Step 10

Reconstitute the vial of chromogenic substrate with 6.5 ml of LRW. Prior to use, an aliquot should be warmed to 37°C ± 1°C.



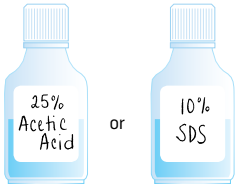
Step 11

At T=10 minutes, dispense 100 µl of pre-warmed chromogenic substrate solution in the same manner as in Step 9. Briefly remove the microplate from the heating block adapter and tap to facilitate mixing. Incubate for 6 minutes.



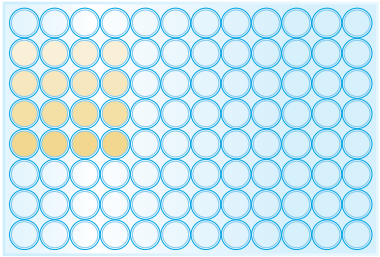
Step 12

At T=16 minutes, dispense 100 µl of stop reagent in the same manner as in Steps 9 and 11. Remove the microplate from the heating block adapter and tap.



Step 13

Read the absorbance of the microplate at 405 – 410 nm.



www.lonza.com/pharmabiotech
www.lonza.com/qcl1000

Materials, Equipment & Documents Needed

Reagents

- Limulus Amebocyte Lysate (LAL) Reagent (QCL-1000™ Reagent)
- Control Standard Endotoxin (CSE)
- Chromogenic Substrate
- LAL Reagent Water (LRW) (# W50-640, W50-100, W50-500)
- Stop reagent (i.e. Acetic acid, 25% v/v glacial acetic acid in water or sodium dodecylsulfate (SDS) solution, 10 g/100 ml in water); reagent does not need to be endotoxin-free and is not included in the kit.

Kits are available in two sizes. 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

- Heating block
- Heating block adapter (# 25-038A)
- Eight-channel pipettor
- Pipettors
- Timer
- Vortex mixer
- Spectro- or Filterphotometer with 405–410 nm filter or microplate reader

Supporting Documents

- Certificate of Analysis (CoA), www.lonza.com/coa
- Limulus Amebocyte Lysate (LAL) QCL-1000™ Package Insert

Points to Consider

- Strictly adhere to incubation times
- Do not use cabinet-style incubators
- 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
- Pre-warm the chromogenic substrate to 37°C ± 1°C prior to use
- Do not vortex the LAL
- Avoid contaminating samples, standards, LRW and accessories
- Equipment should be installed, validated and maintained appropriately

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