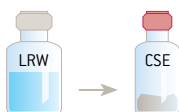


# Endpoint Chromogenic Limulus Amebocyte Lysate (LAL) Assay Procedure Quick Guide

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).



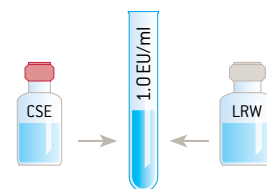
## Step 2

Vortex for 15 minutes.



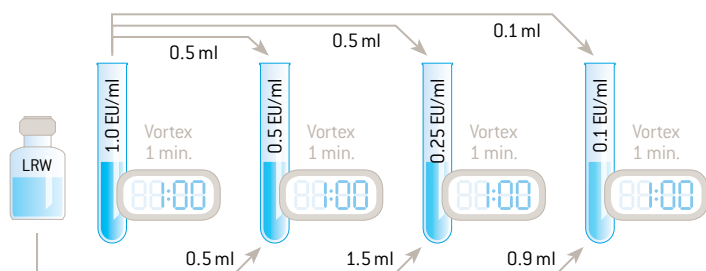
## Step 3

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



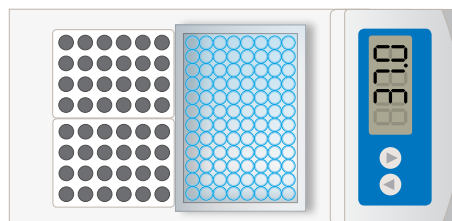
## Step 4

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.



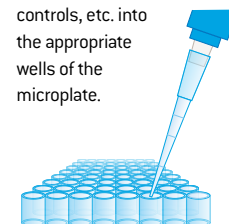
## Step 5

Pre-equilibrate the microplate at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$  in the heating block adapter.



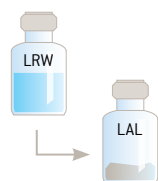
## Step 6

Dispense 50  $\mu\text{l}$  of the LRW blank, endotoxin standards, product samples, positive controls, etc. into the appropriate wells of the microplate.



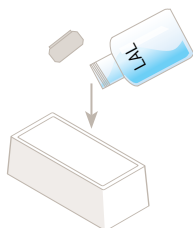
## Step 7

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



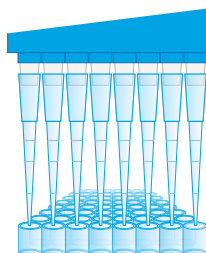
## Step 8

Pour LAL into a reagent reservoir and mix gently.



## Step 9

At time zero ( $T=0$ ), use an eight channel pipettor to dispense 50  $\mu\text{l}$  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.



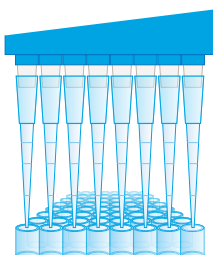
## Step 10

Reconstitute the vial of chromogenic substrate with 6.5 ml of LRW. Prior to use, an aliquot should be warmed to  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .



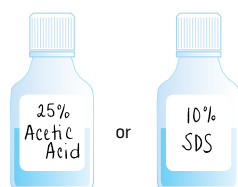
## Step 11

At  $T=10$  minutes, dispense 100  $\mu\text{l}$  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.



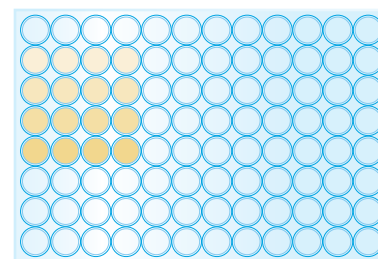
## Step 12

At  $T=16$  minutes, dispense 100  $\mu\text{l}$  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](http://www.lonza.com/pharmabiotech)

[www.lonza.com/qcl1000](http://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](http://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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