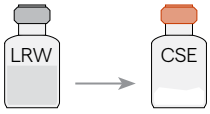
Traditional Gel Clot Limulus Amebocyte Lysate (LAL) Assay Procedure Quick Guide

This is a step by step guide depicting how to perform a traditional gel clot LAL assay. Prior to initiating the assay procedure, allow reagent vials to equilibrate to room temperature.

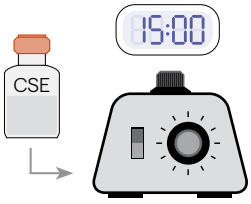
Step 1

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



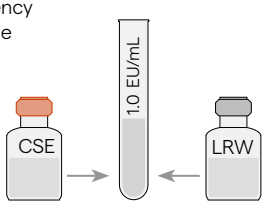
Step 2

Vortex for 15 minutes.



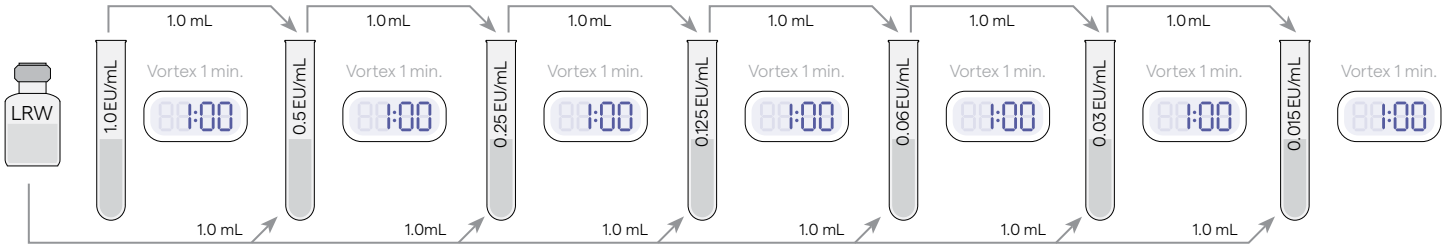
Step 3

Dilute endotoxin with LRW to a concentration of 1.0 EU/mL using the endotoxin potency identified on the Certificate of Analysis (CoA).



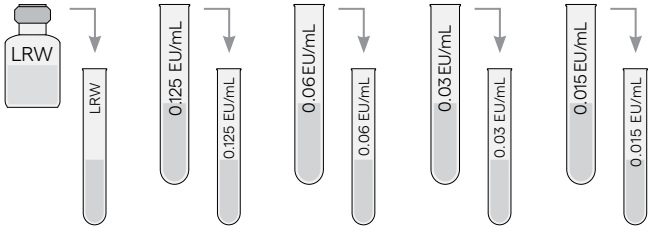
Step 4

Label the tubes with the appropriate endotoxin concentration and add 1.0 mL of LRW to each. Using the 1.0 EU/mL solution, prepare serial 2-fold dilutions to bracket lysate sensitivity.
(Example based on a test using 0.06 EU/mL lysate sensitivity.)



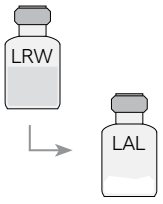
Step 5

Add 100 µL of endotoxin standards and negative control (LRW) into each appropriately labeled 10 × 75 mm reaction tube.



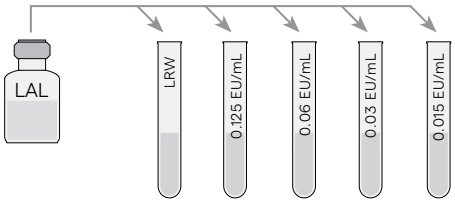
Step 6

Immediately prior to use, reconstitute LAL and gently swirl.



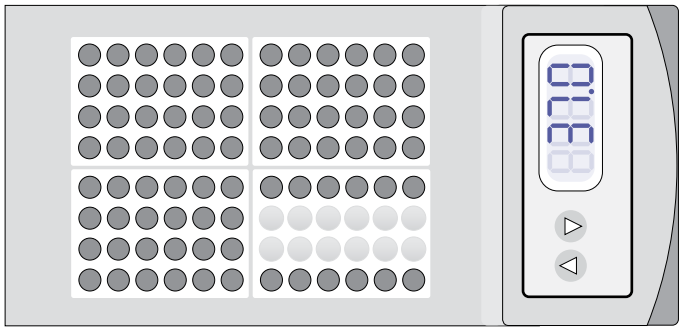
Step 7

Add 100 µL reconstituted LAL into each reaction tube.



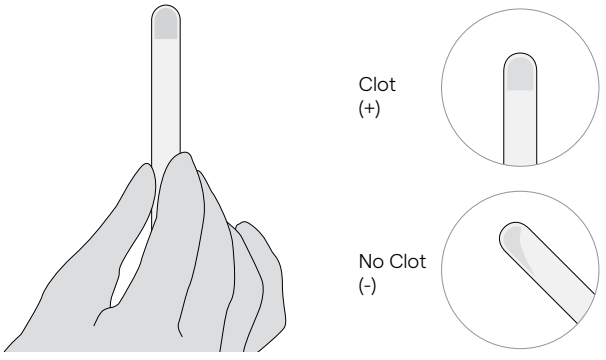
Step 8

Place tubes in 37°C ± 1°C non-circulating water bath or heating block for 60 minutes. Note: Water or dry bath should be located in the lab away from all sources of vibration that could disturb clot formation.



Step 9

After 60 minutes (± 2 minutes) of incubation, carefully remove each tube and invert 180°. Record the reaction in each tube as either positve or negative.



Materials, equipment and documents needed

Reagents

- Limulus Amebocyte Lysate (LAL) Reagent (PYROGENT® Reagent)
- Control Standard Endotoxin (CSE)
- 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, 13 × 100 mm (# N207)
- Glass reaction tubes, 10 × 75 mm (# N201, N205)
- Individually wrapped serological pipettes (optional)
- Tips

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

Equipment and software

- Heating block or non-circulating hot water bath
- Pipettors
- Timer
- Vortex mixer
- Test tube rack

Supporting documents

- Certificate of Analysis (CoA), www.lonza.com/coa
- Limulus Amebocyte Lysate (LAL) PYROGENT® and PYROGENT® Plus 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 contaminating samples, standards, LRW and accessories
- Equipment should be installed, validated and maintained appropriately
- Avoid disturbing the clot

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