SOP: SP020.2
Modified: 1/23/2013
Revised by SOS

LAL Endotoxin Assay

Materials and Reagents:

1. QCL-1000 LAL Endotoxin Kit (BioWhitaker Cat # 50-648U)
2. 200 µl Pipettor
3. 200 µl pyrogen-free, sterile, filter pipet tips
4. 1000 µl Pipettor
5. 1000 µl pyrogen-free, sterile, filter pipet tips
6. Pyrogen free cryovials (Cat# VWR 66021-944)
7. Pyrogen free 15 ml Falcon tubes (Cat# VWR 21008-918)
8. Endotoxin free water
9. Acetic acid
10. Aluminum foil
11. Lab timer
12. 37°C Incubator
13. 37°C Heat block
14. ELISA plate heat block
15. Epoch plate reader, 405 nm filter
16. Vortexer
17. Sterile pyrogen free 96-well assay plate (Cat# VWR 21100-006)

Protocol

1. _____ Remove the vial of purified endotoxin in the LAL Kit from the refrigerator and allow to warm to room temperature (note 1).

2. _____ Suspend the endotoxin in 1 ml of sterile endotoxin free water.

3. _____ Vortex the endotoxin for at least 60 minutes.

4. _____ Record the endotoxin level given in the manual for the LAL Kit, this is the EU level.

5. _____ Prepare 0.1, 0.25, 0.5, and 1.0 EU/ml dilutions of the endotoxin for the standards with endotoxin free water in the pyrogen free cryovials (note 2).

6. _____ Vortex standards for at least 15 minutes. Standards can continue to be vortexed until added to the 96-well plate.

7. _____ Prepare the stop solution with 25% (v/v) acetic acid in endotoxin free water (note 3).

8. _____ Remove a vial of the chromogenic substrate from the LAL Kit.

9. _____ Prepare a small piece of aluminum foil to wrap around the chromogenic substrate vial.

10. _____ Suspend the chromogenic substrate in 6.5 ml of endotoxin free water and wrap the vial in the aluminum foil.

11. _____ Place the chromogenic substrate in the 37°C incubator.

12. _____ Turn on the heat block and put the 96-well plate in it to warm them up.

13. _____ Prepare 200 µl dilutions of the sample to be tested in pyrogen free cryovials using endotoxin free water. These dilutions should be 1:20, 1:100, and 1:500. (notes 5 and 6).

14. _____ Vortex the sample dilutions for at least 15 minutes.
15. _____ Pipet 50 µl of each endotoxin standard into two wells in a sterile 96-well plate (note 4).

16. _____ Pipet 50 µl of each sample dilution into three wells on the 96-well plate.

17. _____ Once the chromogenic substrate and the samples in the 96-well plate have come up to temperature, you can proceed with the rest of the assay.

18. _____ Suspend the Limulus Amebocyte Lysate (LAL) in 3 ml of endotoxin free water, this should be done immediately prior to beginning the actual assay for maximum efficiency (note 7).

19. _____ Beginning with the first dilution of the standard curve, pipet 50 µl of the LAL into the well. Pipet up and down 3 times to ensure proper mixing. It is very important to pipet each well in the same manner to achieve maximum consistency among all wells.

20. _____ Immediately after pipetting the first well, start the lab timer so it begins counting up.

21. _____ Continue pipetting until all wells have been mixed with the LAL substrate.

22. _____ Change the setting on the pipettor to 100 µl.

23. _____ When the timer reaches ~9 minutes, remove the chromogenic substrate from the incubator.

24. _____ When the timer reaches 10 minutes, using the same technique as before, pipet 100µl of the chromogenic substrate into each of the wells.

25. _____ When the timer reaches 16 minutes, using the same technique as before, pipet 100 µl of the stop solution (25% acetic acid) into each of the wells.

26. _____ Read the plate on the Epoch plate reader using the 405 nm filter. (note 8)

27. _____ The readings from the plate reader are given in EU/ml. To calculate the level of endotoxin in the sample, perform the following calculation.

EU/ml x 1 ng/ 10 EU x 1 ml/? mg

This calculation begins with the value from the microplate manager printout
The 1 ng/ 10 EU is a conversion factor to change from EU to ng units
The 1 ml/? mg is the protein concentration from a BCA assay in inverse form.
This calculation will give nanograms endotoxin/milligrams protein as the measurement

Notes:
1. The LAL Endotoxin kit should be stored at 4ºC.
2. The endotoxin standards should also be kept at 4ºC, and are viable for up to two weeks. After that new standards should be prepared.
3. The stop solution is stable at room temperature for many months.
4. Extreme care should be taken when pipetting into the wells of the 96-well plate as to not touch anything but the inside walls of the wells to avoid contamination of the sample within the well.
5. Consistent pipetting is the key to achieving good results with this assay. Make sure to carefully and accurately pipet when making standards, and dilutions of the samples.
6. Generally for recombinant proteins dilutions of 1:10, 1:50, and 1:100 are sufficient.
7. If lysate is frozen at -20ºC immediately after use it can be thawed and used one more time.