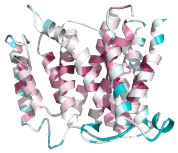
Endotoxin LAL Assay Protocol



Preparing Standards

1. Prepare 1 EU/ml standard
   - Place 0.1 ml of endotoxin stock solution into a 1.7 ml microfuge tube.
   - Add LAL Reagent water.
     Calculate water addition as follows:
     ml water = (X-1) / 10
     x is equal to the concentration of endotoxin in the stock solution.
   - Vortex vigorously for 1 min

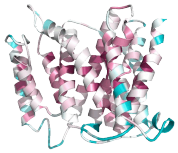
2. 0.5 EU/ ml standard
   - Combine 0.5 ml 1 EU/ml standard and 0.5 ml LAL Reagent water into a 1.7 ml microfuge tube.
   - Vortex vigorously for 1 min.

3. 0.25 EU/ ml standard
   - Combine 0.25 ml 1 EU/ml standard and 0.75 ml LAL Reagent water into a 1.7 ml microfuge tube.
   - Vortex vigorously for 1 min.

4. 0.1 EU/ ml standard
   - Combine 0.1 ml 1 EU/ml standard and 0.9 ml LAL Reagent water into a 1.7 ml microfuge tube.
   - Vortex vigorously for 1 min.

Endotoxin Reaction Protocol

1. Add 50 µl of endotoxin standard or unknown sample to a 1.7 ml microfuge tube.
2. Incubate at 37 °C until temperature equilibrates.
3. At T=0 min, add 50 µl of LAL to the reaction mixture.
4. Vortex vigorously and return to temperature bath.
5. Continue additions to tubes at regular intervals (20 second intervals are needed for this experiment)
6. At T=10 minutes add 100 µl of substrate solution.
   NOTE: The substrate solution should be prewarmed to 37°C
7. Vortex vigorously and return to temperature bath.
8. Continue addition to other tubes at the same time intervals used in Step 5.
9. At T=16 minutes add 100 µl of stop reagent to tube at the same time intervals as steps 5 and 8.
10. Vortex vigorously.
11. Read Absorbance of each reaction tube at 405-410 nm.



Sample Preparation

1. All samples should be run in duplicate.
2. 50 µl of each sample should be added to the appropriate reaction tubes.
3. Samples needed:
   - Blank = 50 µl LAL Reagent water
   - 1 EU/ml standard
   - 0.5 EU/ml standard
   - 0.25 EU/ml standard
   - 0.1 EU/ml standard
   - DNA prepared with ER buffer
     i. Undiluted
     ii. 1:10 dilution
     iii. 1:100 dilution
   - DNA Prepared without ER buffer
     i. Undiluted
     ii. 1:10 dilution
     iii. 1:100 dilution
     iv. 1:1000 dilution
   - Column Elution from with ER buffer
     i. Undiluted
     ii. 1:10 dilution
     iii. 1:100 dilution
   - Column Elution from without ER buffer
     i. Undiluted
     ii. 1:10 dilution
     iii. 1:100 dilution
     iv. 1:1000 dilution

Experimental Notes

1. Experiment will need to be run in two flights.
2. Each flight should include a 1 tube of each for a standard curve and 1 tube of each unknown.
3. Samples are run on a continuous timer.
4. Start running samples at T=0 minutes and make additions appropriately.
   - T=0 begin adding LAL to tubes at 20 second intervals until you have completed the first 19 tubes.
   - At T=10 minutes begin adding substrate to tubes at 20 second intervals.
   - At T =16 minutes begin adding stop buffer to tubes at 20 second intervals.