Protocol 506_Limulus Amoebocyte Lysate modified protocol

Reagents:

- Limulus Amoebocyte Lysate colorometric kit – Lonza
- Endotoxin-free water
- ELISA plates (nunc)
- Samples diluted to an expected range of <1 U/ml LPS (i.e. use samples to expect to be very clean neat. Samples with known bacterial contamination e.g. cecal content, serum from mice injected with bacteria, should be measured in 5-fold serial dilutions starting at 1:100)

Method:
As for protocol supplied with kit, but with all volumes halved to increase number of samples per kit. The first incubation time is increased (similar to their suggested high sensitivity test protocol), which seems to compensate for lower absorbances with this reduced volume

1. Rehydrate the assay reagents using endotoxin-free water and pre-warm to 37ºC in a bacterial incubator for roughly 1hr.

2. Use 96 well tissue culture plate (flat bottomed). Keep incubated (37 degrees) at all times.

3. Using endotoxin-free (filter) tips, plate 25ul/well samples plus blank and standards (supplied in kit), all in duplicate in pre-warmed plate:

    a. 1, 0.5, 0.25, 0.1U/ml Endotoxin (from kit) in LPS free water supplied plus blank (water only)

4. Add 25ul/well Limulus lysate; pipette to mix (i.e. one tip per well).

5. ---Incubate at 37 degrees for 16 mins---

6. Add 50ul/well chromogenic substrate.

7. ---Incubate at 37 degrees for 6 mins---

8. Stop reaction with 50ul/well stop solution (10% SDS or 1% acetic acid).

9. Read A405 with microplate reader.

Notes
With this modified protocol, the standard curve was not linear as suggested in origional protocol, but the test was still able to detect 0.1U/ml with confidence.

e.g.