Measuring endotoxin content of microparticles

Day 0 –

1. Add 1-1.5 mg of particles to a sterile 1.5 mL microcentrifuge tube
2. Suspend at 1 mg/mL in sterile molecular biology-grade water. Vortex, rake, and sonicate to ensure adequate suspension.
    a. Also include a "water only" control
    b. Do particles in duplicate
3. Store in cold room overnight.

Day of assay – Do all steps inside the TC hood to keep things sterile

- Spin particle tubes at max speed for 20 minutes in cold room centrifuge to pellet
- Use supernatant for endo screening

Prepare materials:

Endotoxin standard solution – Reconstitute lyophilized powder with 1 mL sterile water. The number of units is printed on the tube. Vortex vigorously for 15 minutes. Standard is stable for 4 weeks at 2-8 C. If pulling from fridge, prior to use, warm to RT and vortex for 15 minutes.

Dilute the standard solution with sterile water. Vortex each solution for 1 minute before adding to next solution.

Standard (units/mL)	µL previous	µL sterile water
1	Variable	Variable
0.5	250	250
0.25	250	750
0.1	100	900
0	0	500

LAL solution – Reconstitute lyophilized powder with 1.7 mL sterile water and swirl gently to dissolve. Do not vortex. LAL is stable for 1 week at –20 C. May be thawed only once.

Chromogenic substrate – Reconstitute lyophilized powder with 3.4 mL sterile water to a yield a final concentration of 2 mM. Substrate is stable for 4 weeks at 2-8 C. Prior to use, warm to 37 C.

Use barrier tips and work in TC hood throughout.

Assay procedure

1. Pre-equilibrate a TC-treated flat bottom 96-well plate at 37 C for ten minutes by putting it on the right two blocks of a bench-top heater.

2. Transfer the plate and a 37 C heating block to the TC hood. Carefully add 50 uL of each standard and sample solution to the plate. **Do each sample in duplicate, and each standard in triplicate**.

3. At T=0, use a sterile reagent reservoir and a multi-channel to add 50 uL LAL per well. Tap mix and transfer back to heating block for 10 minutes.

4. After 10 minutes, transfer plate and a 37 C heating block to TC hood. Use a sterile reagent reservoir and multi-channel to add 100 uL pre-warmed Chromogenic Substrate to each well. Tap mix and transfer back to heater for 6 minutes.

5. At T=30 minutes (or when the standard curve has a consistent color gradient), use a non-sterile reagent reservoir and multi-channel to add 100 uL of 25% acetic acid (diluted in milli-Q water) while plate is on heater. Remove from heater and tap mix several times.

6. Measure absorbance at 405-410 nm.
    a. Also measure simultaneously at 350 nm