EAA™ (Endotoxin Activity Assay) 
Instructions for Use

Prior to starting the EAA™ ensure that the Berthold SmartLine luminometer is “ON” and the heating incubator shaker is “ON” and warmed to the setting of 37°C +/- 1.0°C.

**SETUP**

1. Setup the EAA™ tubes for each patient sample in the supplied tube racks and remove the caps.
   *Setting up the tubes in the supplied rack the same time every way helps to keep you familiar with the assay. Be sure to “push” the caps off of the tubes to avoid tubes breaking*

2. Combipipette 1.0 mL of the EAA Reagent into Tubes 1, 2 and 3 (in duplicate).
   *Pipette down the side of the tube to avoid solution splashing back up.*

**SAMPLE**

3. After mixing the patient blood sample by gentle inversion, pipette 0.5mL aliquots directly into the Aliquot Tube and the LPS MAX Tube using a sterile pipette tip. Vortex the LPS MAX tube.
   *Mix the sample by inverting 20 times. Vortex the LPS Max tube for 10 full seconds.*

4. Place the rack(s) in the incubating shaker, close the lid and incubate for 10 minutes.

**ALIQUOT**

5. Following the 10-minute incubation, open the lid, vortex the Aliquot tube then combipipette 40μL of blood into Tubes 1 and 2 (in duplicate) using a sterile combipipette tip.
   *If using the incubator timer be sure to turn the incubator back on when the timer goes off. Pipette blood down the sides of the tubes being sure to touch the tip to the inside of the tube to avoid drips.*

6. Vortex the LPS Max Tube, then combipipette 40μL of blood into Tubes 3 (in duplicate) using a sterile combipipette tip.
   *Pipette blood down the sides of the tubes being sure to touch the tip to the inside of the tube to avoid drips.*

**LPS MAX**

7. Vortex Tubes 1, 2 and 3 and place back into the rack(s) in the incubating shaker, close the lid and start the motion (set at 100rpm) and the timer (set at 14 minutes).
   *Vortex each duplicate at one time. Do this with two hands and ensure that all tubes are being vortexed for the same amount of time.*

**RESULTS**

8. Insert the EAA labeled chipcard into the luminometer and press Start.
   *Do this only after the incubation period has ended.*

9. Following the 14-minute incubation, follow the instructions on the luminometer display for reading of the EAA tubes in the proper order.

10. Vortex each tube gently, open the sample drawer and place the tube in the sample holder, close the sample drawer and wait for an RLU reading. Repeat for the next tube.
    *Try to vortex each tube for the same amount of time before putting them in the smartline for reading.*

11. After all tubes are read the EAA results are printed automatically on the attached printout.

12. Repeat for each patient sample if applicable.

TECHNICAL SUPPORT: 1-888-426-4264 Option 2.

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Additional instructions and guidance are available on the EAA™ Endotoxin Activity Assay product documentation and support resources.