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Porcine LAL Chromogenic Endotoxin (LPS) Quantitation Kit

(50 assays)

Catalog number: BG-POR10895

The kit is designed to quantitatively detect the levels of porcine endotoxin in serum, plasma and other suitable sample solution.

Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- Accurate pipetting is critical for maintaining consistent results. A repetitive pipettor can aid in normalizing volumes between samples. Ensure pipetting order and rate of reagent addition remain consistent from well to well and row to row.
- All materials (e.g., pipette tips, glass tubes, microcentrifuge tubes and disposable 96-well microplates) must be endotoxin-free.
- Adjust the sample pH to 6-8 using endotoxin-free 0.1M NaOH or 0.1M HCl. Avoid pH-electrode contamination of the sample by testing the pH of a small sample taken from the bulk sample.
- Components of undiluted serum interfere in the assay. Serum samples must be diluted 50- to 100-fold to be compatible. The serum must be completely free of RBCs, and the diluted sample may need to be heat-shocked (70°C for 15 minutes).
- Store samples to be tested to stop all bacteriological activity. Store at 2-8ºC for 24 hours.
- Maintaining the correct temperature is critical for reproducibility. Use a proper heating block at 37ºC±1ºC. Do not use a cabinet-style incubator to perform the assay. • Endotoxin adheres to glass and plastic surfaces; before pipetting, vortex solutions to ensure the correct endotoxin concentrations are measured.
- Glass tubes are preferred for making standard stock solutions; however, polystyrene or polypropylene microcentrifuge tubes (1.5 mL) may also be used. When using microcentrifuge tubes, dedicate the bag of tubes for the assay and follow aseptic techniques.
- If the test sample endotoxin concentration is >1.0 EU/mL, dilute the sample five-fold in endotoxin-free water.

Intended use

The kit is used to quantify the levels of Lipopolysaccharides.

Standard Range	0.1 – 1.0 EU/ml
Assay time	31 min
Validity	Six months
Store at	2-8 °C

Assay principle

LAL Chromogenic Endotoxin (LPS) Quantitation Kit offered by Novatein Biosciences is an efficient, quantitative endpoint assay for the detection of lipopolysaccharides. The principle is based on the activation by Bacterial endotoxin, of a proenzyme in the Limulus Amebocyte Lysate (LAL). The activated proenzyme then catalyzes the hydrolysis of p-Nitroaniline (pNA) from the colorless substrate, Ac-Ile-Glu-Ala-Arg-pNA; the activation rate is proportional to the endotoxin concentration in the sample. The released pNA is photometrically measured at 405-410 nm after stopping the reaction.

Materials supplied

1. LPS Standard (Endotoxin from E.coli, 011:B4): 1 vial
2. Limulus Amebocyte Lysate (LAL), lyophilized: 2 vials
3. Chromogenic Substrate: 1 vial
4. Endotoxin-free Water: 30 ml
5. Package insert 1

Materials required but not supplied

- Disposable endotoxin-free glass tubes or 1.5mL microcentrifuge tubes.
- Disposable endotoxin-free pipette tips.
- Disposable endotoxin-free 96-well microplates.
- Heating block at 37ºC.
- 25% acetic acid (Stop Solution).
- Multichannel pipette.
- Plate reader.

Reagent Preparation

Preparation of Endotoxin Standard Stock Solutions

- Each Standard vial contains ~15-40 EU of lyophilized LPS. Actual concentration is printed on the vial label.
- Reconstitute by adding 1 mL of room temperature endotoxin-free water to make LPS Standard Stock. For example, a vial with a concentration of 30 EU, when reconstituted with 1.0 mL of endotoxin-free water, will yield a concentration of 30 EU/ml.
- Shake the solution vigorously for at least 15 minutes on a vortex mixer before use
- Dilute the LPS Stock solution to working standard solutions of 1.0, 0.5, 0.25 and 0.1 EU/ml
- Vortex the diluted standard solutions vigorously for 1 minute before use
- Reconstituted stock solution is stable for 4 weeks at 2-8 ^{o}C

Preparation of Limulus Amebocyte Lysate (LAL) working solution

The LAL reagent contains lyophilized lysate prepared from the circulating amebocytes of the horseshoe crab Limulus polyphemus. Reconstitute immediately before use with 1.4 mL of endotoxin-free water and swirl gently to dissolve the powder. **Avoid foaming; do not vortex the solution.**

Store lyophilized LAL away from direct light at 2-8 ^{o}C. Reconstituted reagent could be stored at -20 ^{o}C for upto 1 week, if frozen immediately after reconstitution. Once thawed, gently mix the reagent by swirling before use and do not store it again.

Preparation of Chromogenic Substrate

Each vial contains ~7 mg of lyophilized substrate. Reconstitute the Chromogenic Substrate by adding 6.5 mL of endotoxin-free water to yield a final concentration of ~2mM. Reconstituted substrate is stable for 4 weeks at 2-8 ^{o}C. Protect from light.

Assay Procedure

Bring all reagents to room temperature before use. Endotoxin (LPS) Standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of endotoxin (LPS) concentration in samples.

1. Equilibrate the microplate in a heating block/ incubator for 10 minutes at 37ºC
2. With the microplate maintained at 37ºC, carefully dispense 50 µL of each standard or unknown sample into the appropriate microplate well, cover the plate with the lid and incubate for 5 minutes at 37ºC.

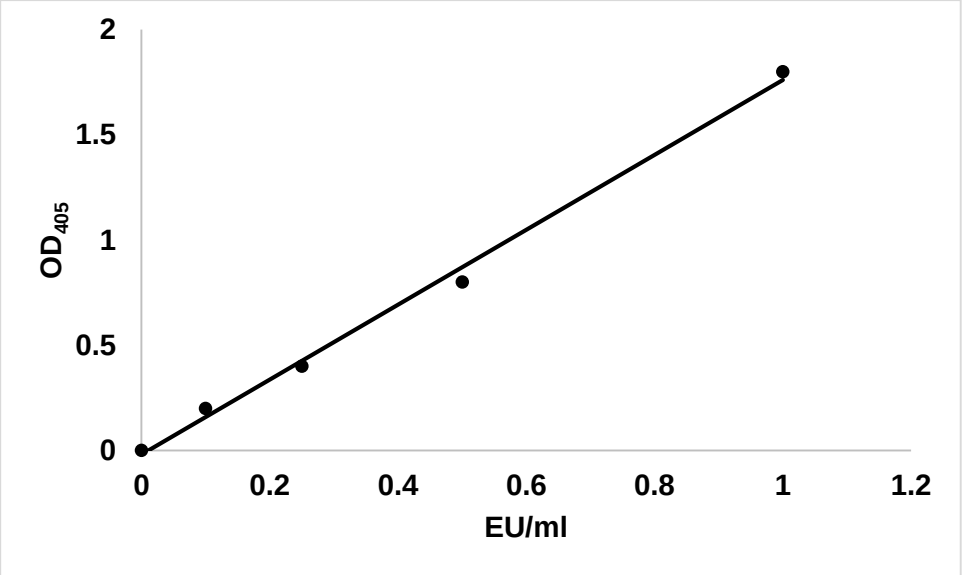
   Note: We recommend that each standard solution and each sample is measured in duplicate. The blank contains 50µL of endotoxin-free water
3. Add 50 µL of LAL to each well using a pipettor, cover the plate with the lid and gently shake the plate on a plate shaker for 10 seconds. Incubate the plate at 37ºC for 10 minutes.
4. After exactly 10 minutes, add 100µL of substrate solution to each well. Pipette the substrate solution in the same manner as in step 3. Maintain a consistent pipetting speed.
5. Cover the plate with lid and gently shake on a plate mixer for 10 seconds. Incubate the plate at 37ºC for 6 minutes.
6. Add 50µL of Stop Reagent (25% acetic acid). Maintain the same pipetting order as in steps 3 and 4. Gently shake the plate on a plate mixer for 10 seconds.
7. Read the Optical Density (O.D.) at 405-410 nm using a microtiter plate reader immediately.

Result calculation

For calculation, (the relative O.D.410) = (the O.D.410 of each well) – (the O.D.410 of Zero well). The standard curve can be plotted as the relative O.D.410 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human LPS/ endotoxin concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution

Typical Standard Curve



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