Endotoxin Detection and Removal System FAQs

Updated June 2014

General Questions

Q1. Which endotoxin detection kit should I use?

GenScript endotoxin detection kits meet various experiment requirements.
ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat. No. L00350) utilizes chromogenic LAL assay for accurate *in vitro* end product endotoxin detection in a broad range (0.005–1 EU/ml). Test sample should be Colorless and Clear liquid.
ToxinSensor™ Gel clot Endotoxin Assay Kit (Cat. No. L00351) is intended for convenient qualitative *in vitro* end product endotoxin test based on gelation principle.
ToxinSensor™ Single Test Kits (Cat. No.L00447-L00451) is designed for one-step qualitative *in vitro* end product endotoxin test. The kit use gelation principle under different sensitivities (0.015 EU/ml, 0.03 EU/ml, 0.06 EU/ml, 0.125 EU/ml and 0.25 EU/ml).

Q2. What equipment do I need to run my assay?

Different equipments are required if you are running one of our endotoxin detection kits. **ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat. No. L00350)** requires a color spectrophotometer; **ToxinSensor™ Gel clot Endotoxin Assay Kit (Cat. No. L00351)** only requires a 37 °C water bath, and the visible gel formation is present in final results.

Sample Preparation

Q3. What pH should I set my sample at? What should I adjust it with?

The pH of the sample should be at pH 6-8 to ensure good linearity. Consequently, we recommend adjusting pH value using solidium hydroxide (0.1 N, dissolved in LAL reagent water) or hydrochloric acid (0.1 N, dissolved in LAL reagent water) if necessary.

Q4. Why is it important to vortex standard endotoxin solution?

Endotoxin can adhere to the surface of glassware, yet this problem can be mediated by vortexing. The endotoxin standard solution should be vigorously vortexed for 15 minutes prior to making dilutions, and repeat the vortex step each time before making dilutions, not just after reconstitution. Each standard endotoxin dilution should be vortexed for at least one minute prior to using.

Q5. Can I use plastic ware when I prepare standard endotoxin solution?

Endotoxins adhere strongly to glassware and are difficult to remove completely during washing. Standard laboratory autoclaving procedures have little or no effect on endotoxin levels. Heating glassware at 180°C overnight is recommended to destroy any attached endotoxin molecules. We recommend you to only use new plasticware tubes which are certified to be endotoxin-free when you prepare standard endotoxin solution. GenScript supplies **ToxinSensor™ Endotoxin-free Tubes (Cat. No. M01072)** for you to dilute or aliquot samples. Endotoxin adheres to plastic surfaces more strongly than glass surfaces.

Q6. Is standard endotoxin solution reusable after developed (color formed)?

No. Standard endotoxin solution should be prepared fresh for each test.

Application

Q7. What types of samples are not compatible with ToxinSensor™ Endotoxin Detection System?

GenScirpt endotoxin detection kits are not intended to detect endotoxin in the diagnosis of human disease, clinical samples (e.g. blood or blood products, serum, antibiotic sample etc.), patient management, and cell/bacterial culture medium. Besides, because β-glucans can interfere non-specifically with LAL reagents and cause a false positive result, we do not recommend using our Endotoxin Assay Kits to detect β-glucans samples or samples contaminated by β-glucans.

Q8. How to run an endotoxin detection assay for medical devices?

Infuse 15 ml of endotoxin-free water into the lumen of medical device (e.g. infusion apparatus, dialysis tubing, *etc.*) and shocks five times, seal all the ends and incubate it at 37°C water bath for 2 hours, then transfer the water to an endotoxin-free vial. Detect the endotoxin concentration in the water by using **ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat. No. L00350)**, and the total endotoxin value in the water can be determined.

Result Analysis

Q9. How to determine the reliability of the final data?

Firstly, we recommend you to prepare all materials included in the kit in a laminar flow cabinet and avoid contamination during assay process.
For **ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat. No. L00350)**, if the OD value of your sample is in the range of the standards, the final data will be reliable after you obtain a good linearity

(R≥0.980).

For **ToxinSensor™ Gel Clot Endotoxin Assay Kit (Cat. No. L00351)**, you can obtain the reliable final result through negative and positive controls. If you get a positive result in the negative control, it may indicate that the LAL or LAL reagent water has been contaminated, or if you get a negative result in the positive control, it may indicate that the LAL reagent has lost activity.

Q10. How long should I wait to measure the OD value using ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat. No. L00350)? How long will the color remain stable using the kit?

You can wait 5 minutes to measure the OD value after adding Color-stabilizer #3. The color will be stable for 1-2 hours.

Q11. Why the negative blank shows an increase in OD value using ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat. No. L00350)?

The endotoxin-free materials and the LAL reagent water supplied with **ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat. No. L00350)** have a very low endotoxin level (about <0.0015 EU/ml), furthermore, the Color-stabilizer #3 has a similar color as the final color. Consequently, the above factors may result in an increased OD value in the negative blank.

Q12. Can I use 96-well microplate to read the absorbance of each reaction at 545 nm?

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat. No. L00350) is designed to deliver high sensitivity quantitative assay in tubes, and not intended to be used for a 96-well plate assay. However, you can perform the assay in tubes, after color development, transfer 200 µl of the final solution into an endotoxin-free 96-well plate to read the result.