ToxinSensor™ Single Tests Kit

Cat. No. L00448, L00449, L00450, L00451

Version: 12172020

Index

I. DESCRIPTION

GenScript **ToxinSensor™ Single Tests Kit** is designed to be a qualitative *In Vitro* end-point endotoxin test for human and animal parenteral drugs, biological products, and medical devices.

Limulus Amebocyte Lysate (LAL) as supplied is to be reconstituted with sample or control directly. After incubation for 1 hour, and in the presence of endotoxin, gelation occurs; in the absence of endotoxin, gelation does not occur.

GenScript supply a series of ToxinSensor™ Single Tests Kits with different sensitivity (0.03 EU/ml, 0.06 EU/ml, 0.125 EU/ml and 0.25 EU/ml).

When using the ToxinSensor™ Single Test Kit, you just need to add 200µl of sample or control to LAL, then, wait for the required incubation time to allow for gel to form. There is no need to reconstitute the system with LAL Reagent Water before use.

Note: Our kit is used for testing samples that are certified free of Beta-Glucans contaminant. This contaminant can come from yeast, bacteria, and cellulosic materials, such as blood products.

II. KEY FEATURES

- High reproducibility
- Broad detection range
- Easy to use

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

III. PRODUCT INFORMATION

Product Name	Cat. No.	Size	Sensitivity
ToxinSensor™ Single Test Kit	L00448-20 L00448-40	20 Assays 40 Assays	0.03 EU/ml
	L00449-20 L00449-40	20 Assays 40 Assays	0.06 EU/ml
	L00450-20 L00450-40	20 Assays 40 Assays	0.125 EU/ml
	L00451-20 L00451-40	20 Assays 40 Assays	0.25 EU/ml

Each kit contains a designated number of vials of 20 or 40 containing lysate prepared from the circulating amebocytes of the horseshoe crab (*Limulus polyphemus*) standardized to detect the labeled concentration (EU/ml) of the FDA Reference Standard Endotoxin.

IV. MATERIALS AND EQUIPMENT NOT PROVIDED

1. Sodium hydroxide, 0.1 N, dissolved in LAL reagent water. The reagent is for pH adjustment.
2. Hydrochloric acid, 0.1 N, diluted in LAL reagent water. The reagent is for pH adjustment.
3. Water bath or heating blocks set at 37 °C ± 1.0°C
4. Vortex mixer
5. Endotoxin Standard (Needs to match the LAL batch)
6. LAL Reagent water
7. Pipettes, 0.2 ml and 1.0 ml, endotoxin-free
8. Endotoxin-free vials
9. Timer

V. ENDOTOXIN DETECTION PROTOCOL

To avoid contamination, conduct the experiment in a laminar flow cabinet at room temperature in a designated Reagent Preparation Area; wear disposable gloves and use endotoxin-free materials in order to avoid contamination.

1. Sample Preparation

A. pH adjustment

The pH of the sample should be at pH 6-8 (18-26℃) to ensure good linearity. We recommend adjusting the pH with 0.1 N HCl or NaOH if needed.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

B. Dilution

When the estimated endotoxin level in a sample is out of the detection range, the sample needs to be diluted before detection. The dilution factor is determined by MVD*. For best performance, do not exceed the MVD of your sample.

***MVD** (Maximum Valid Dilutions): a dilution factor calculated by endotoxin limit (in EU/ml) divided by lambda. Lambda is the labeled sensitivity of LAL reagent. We provide LAL reagents with different sensitivities. For specific sensitivity, please refer to the label on the kit.

For example, if the endotoxin limit is 10,000 EU/ml, and lambda (the reagent's labeled sensitivity) of LAL test is 0.25EU/ml (L00451-20 or L00451-40), then MVD should be calculated as: 10,000/0.25 = 40,000.

2. Preparation of Positive Controls.

Note: This step could be skipped if a simple & quick screening is preferred.

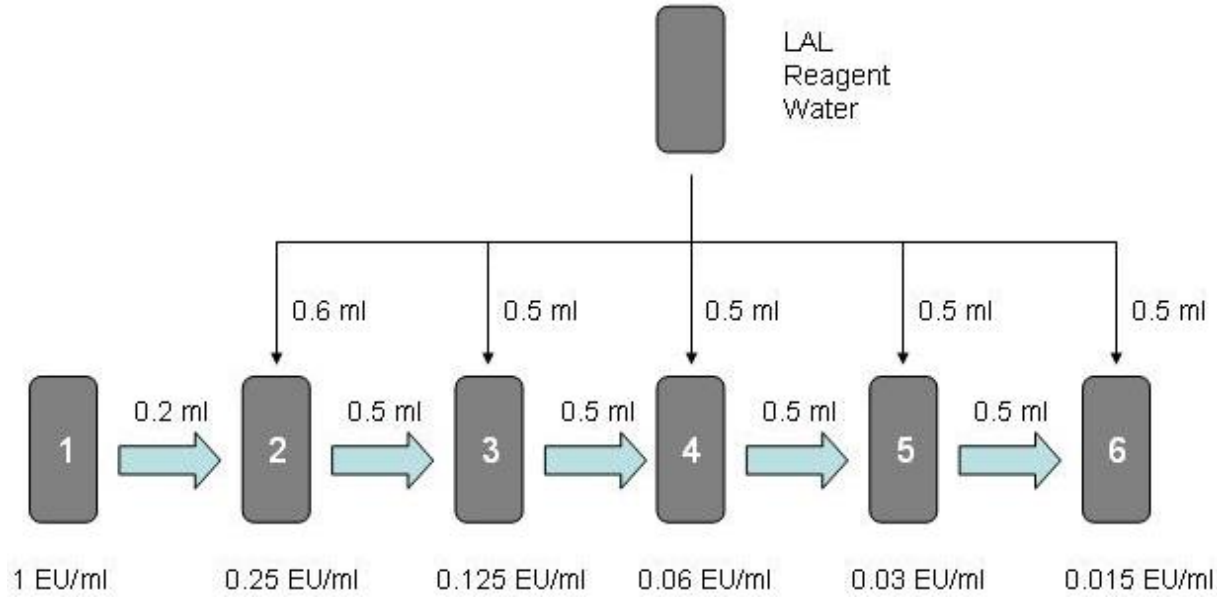
A. Reconstitute endotoxin standard according to the label with 1-2mL LAL Reagent Water.

B. Vortex the vial of endotoxin standard for at least 15 minutes to get a stock solution.

C. Dilute the stock solution with LAL Reagent Water to a concentration of 1 EU/ml. Each dilution should be vortexed for 60 seconds prior to proceeding to the next dilution.

D. Prepare a serial of dilutions from the 1 EU/ml endotoxin solution as shown in the following chart.

E. Positive control is at least 2 lambda concentration of endotoxin, e.g., if the sensitivity of the kit is 0.125EU/ml, use an endotoxin positive control at least at 0.25EU/ml.



3. Negative Control

LAL Reagent Water may be used as a negative control.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

4. Test Procedure

A. The vials containing LAL serve as the test containers. Before use, bring all the contents of the vial together by gently tapping the bottom of the vial on a hard surface. Remove the rubber stopper carefully to avoid microbial and endotoxin contamination.

B. Carefully transfer 0.2 ml of positive control (if prepared), negative control, and the test samples to the LAL vials. Cap the vials and mix them thoroughly.

C. Incubate all vials at 37°C ±1°C in a water bath or heating block set. Keep racks standing upright while incubating.

D. Remove the rack after 60 ± 2 minutes of incubation. Invert each vial and check whether a gel has formed or not. Do not shake vigorously while checking; it will break up the clotted gel.

a) A positive reaction is characterized by the formation of a firm gel that remains intact when inverting the vial upside down.

b) A negative reaction is characterized by the absence of a solid clot. The lysate may show increased turbidity or viscosity. This is considered a negative result.

E. Calculate the endotoxin level: the endotoxin level in the positive sample is equal or higher than the detection sensitivity of LAL kit used, while in the negative sample it is lower than the detection sensitivity of LAL kit applied.

VI. EXAMPLE

1. Sample: Protein A (1 mg/ml in PBS, pH 7.4) is purified from *E. coli* lysate by Ni-NTA Resin.
2. Make dilutions using LAL Water: 1: 200,000, 1: 400,000, 1: 800,000
3. The test is performed as described above and the assay result is listed below:

Positive control	Negative control	1: 200,000	1: 400,000	1: 800,000
+	-	+	-	-

4. Endotoxin concentration in this sample is: from 200,000×0.25 to 400,000×0.25, so that is, from 50,000 to 100,000 EU/ml.(0.25 EU/ml is the lambda of the kit)

VII. TROUBLESHOOTING

Problem	Possible Cause	Suggestions
A gel formed in the negative control	The materials (e.g. tips, vials, water etc.) may be contaminated.	Pay more attention to operation and keep the assay under laminar flow cabinet.
No gel formed in the positive control	1. The endotoxin standard is not mixed well. 2. The endotoxin standard does not match the LAL batch. 3. The potency of endotoxin standard decreased for incorrect storage conditions or frequent freezing and thawing.	1. The standard should be vigorously vortexed for 15 minutes prior to use. 2. Use the standard matching the batch of LAL. 3. Prepare a new endotoxin standard.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

VIII. ORDERING INFORMATION

Product Name	Cat. No.
ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit	L00350
ToxinSensor™ Gel Clot Endotoxin Assay kit	L00351
ToxinEraser™ Endotoxin Removal kit	L00338

For *In Vitro* Research Use Only.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com