ToxinSensor™ Single Tests Kit with Standard

Cat. No. L00856, L00857,L00858,L00859

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I. DESCRIPTION

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

Tachypleus Amebocyte Lysate (TAL) 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 Standard with different sensitivity (0.03 EU/ml, 0.06 EU/ml, 0.125 EU/ml and 0.25 EU/ml).

When using the Kit, you just need to add 200µl of sample or control to TAL, then, wait for the required incubation time to allow for gel to form. There is no need to reconstitute the system with TAL 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

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III. PRODUCT INFORMATION

Product Name	Cat. No.	Size	Sensitivity
ToxinSensor™ Single Test Kit with Standard	L00856-20	20 Assays +1 bottle of Standard	0.03 EU/ml
	L00856-40	40 Assays +1 bottle of Standard	
	L00857-20	20 Assays +1 bottle of Standard	0.06 EU/ml
	L00857-40	40 Assays +1 bottle of Standard	
	L00858-20	20 Assays +1 bottle of Standard	0.125 EU/ml
	L00858-40	40 Assays +1 bottle of Standard	
	L00859-20	20 Assays +1 bottle of Standard	0.25 EU/ml
	L00859-40	40 Assays +1 bottle of Standard	

Each kit contains a designated number of vials of 20 or 40 containing lysate prepared from the circulating amebocytes of the horseshoe crab (*Tachypleus tridentatus*) 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 TAL reagent water. The reagent is for pH adjustment.
2. Hydrochloric acid, 0.1 N, diluted in TAL 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. TAL Reagent water
6. Pipettes, 0.2 ml and 1.0 ml, endotoxin-free
7. Endotoxin-free vials
8. 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.

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.

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***MVD** (Maximum Valid Dilutions): a dilution factor calculated by endotoxin limit (in EU/ml) divided by lambda. Lambda is the labeled sensitivity of TAL reagent. We provide TAL 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 TAL test is 0.25EU/ml (L00859-20 or L00859-40), then MVD should be calculated as: 10,000/0.25 = 40,000.

2. Preparation of Positive Controls.

Note: The standard provided in this kit is matched to the lot therefore should be used only with this kit.

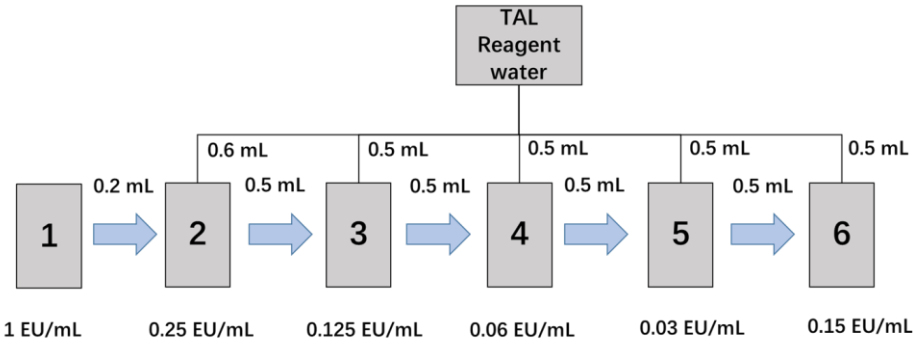
A. Reconstitute endotoxin standard according to the label with 1-2mL TAL Reagent Water. The reconstituted endotoxin stock solution is stable for 1 week if store at 2-8℃ and 4 weeks if store at -20℃.

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

C. Dilute the endotoxin with TAL 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 concentrations 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

TAL Reagent Water may be used as a negative control.

4. Test Procedure

A. The vials containing TAL 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, negative control and the test samples to the TAL 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.

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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 the TAL kit used, while in the negative sample it is lower than the detection sensitivity of the TAL 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 TAL 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 was not mixed well. 2. The endotoxin standard does not match the TAL 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 TAL. 3. Prepare a new endotoxin standard.

For *In Vitro* Research Use Only.

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