ToxinSensor™

Endotoxin Detection System

Version 12172010

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User Manual

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Intended Use

GenScript ToxinSensor™ Endotoxin Detection System is intended for use as an *in vitro* end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices. The system is not intended for use in the detection of endotoxin in a licensed reagent, clinical samples or the diagnosis of human disease. A measurable endotoxin concentration range of 0.005 to 1 EU/ml can be achieved.

Warning

For *In Vitro* Diagnostic Use Only. Not intended to detect endotoxemia in man or animals, or for use in clinical diagnosis, patient management, cell bacterial culture medium, serum, blood or blood products,

Background

For biopharmaceutical companies, endotoxin detection is the most critical quality control test to ensure that manufacturing of pharmaceutical products are free of endotoxin contaminations. There are three LAL test methods, and the following is a general selection guide to help you decide which method to use:

Methods	Maximum Sensitivity	Regulatory Requirements
Gel-clot	0.03 EU/ml	Non-circulating water bath or dry bath incubator
Chromogenic	0.005 EU/ml	A microplate reader (an incubating reader is required for the kinetic method)
Turbidimetric	0.001 EU/ml	An incubating microplate reader

After checking factors, such as budget, amounts of protein sample, test frequency, and assay sensitivity, you can select one endotoxin detection kit from GenScript:

Cat. No.	Size	Test	Methods	Sensitivity
L00350C	16 rxns	Quantitative	Endpoint	0.005 to 1 EU/ml
L00350	32 rxns		Chromogenic	
L00351	40 rxns	Semi-quantitative	Gel-clot	>0.25 EU/ml

Product Overview

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit

This kit is designed as a qualitative test that is simple and sensitive for detection of the presence of lipopolysaccharides in samples. It uses a colorimetric method in which endotoxin catalyzes the activation of a proenzyme in LAL, which will cleave a colorless substrate to produce a colored end-product. The end-product can be measured spectrophotmetrically and compared to a standard curve.

- Good linearity and good reproducibility
- High sensitivity and board application range
- Ready-to-use reagents and materials, such as endotoxin-free tips, endotoxin-free tubes, *etc.*

Kit Contents

PK		Label	Volume
L00350	L00350C		
2 bottles	1 bottle	LAL Reagent Water	50 ml
2 vials	1 vial	Limulus Amebocyte Lysate (LAL)	-
2 vials	1 vial	*E. coli* Endotoxin Standard	6 EU
2 vials	1 vial	Chromogenic Substrate	-
1 bottle/50 ml	1 bottle/10 ml	Buffer S for Color-stabilizer #1	50 ml
2 vials	1 vial	Color-stabilizer #1	-
2 vials	1 vial	Color-stabilizer #2	-
2 vials	1 vial	Color-stabilizer #3	-
6 x 8 vials	3 x 8 vials	Endotoxin-free Vials	-
1 box (96 tips)	1 box (96 tips)	Endotoxin-free Tips	200 µl
2 bags (12 tips)	2 bags (12 tips)	Endotoxin-free Tips	1000 µl
1	1	Incubation Rack	-

Product Overview, continued

Materials and equipments not provided
1. Sodium hydroxide, 0.1 N, dissolved in LAL reagent water. The reagent is for adjustment of the pH of samples if required.
2. Hydrochloric acid, 0.1 N, diluted in LAL reagent water. The reagent is for adjustment of the pH of samples if required.
3. Oven set at 37°C ± 1.0°C.
4. Spectrometer or filter photometer with a 545 nm filter.
5. Vortex mixer

Kit Storage

The kit should be stored dry at room temperature for up to one month. For longer storage, the kit can be kept at 2–8°C for up to one year. Do not freeze the kit or any of its components.

Ordering Information

Cat. No.	Product	Quantity	Price
L00350C	ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit	16 rxns	$80.00
L00350	ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit	32 rxns	$150.00

Product Overview, continued

ToxinSensor™ Gel Clot Endotoxin Assay Kit

This kit is designed to be the simplest semi-qualitative test for gram-negative bacterial endotoxin that conforms to FDA Guideline. Similar performance requirements for gel clot assays have been published and are updated regularly in the United States Pharmacope.

- Good reproducibility
- Competitive price
- Ready-to-use reagents and materials, such as endotoxin-free tips, endotoxin-free tubes, *etc.*

Kit Contents

PK	Label	Volume
4 bottles	LAL Reagent Water	10 ml
2 vials	Limulus Amebocyte Lysate (LAL)	2 ml
2 vials	*E. coli* Endotoxin Standard	0.5 EU
5 x 16 vials	Endotoxin-free Vials	-
1 box (96 tips)	Endotoxin-free Tips	200 µl
2 bags (12 tips)	Endotoxin-free Tips	1000 µl
1	Incubation Rack	-

Product Overview, continued

Materials and equipments not provided
1.Sodium hydroxide, 0.1N, or hydrochloric acid, 0.1N dissolved in LAL Reagent Water, for pH adjustment of samples if necessary
2. Oven or non-circulating hot water bath (37 ± 1°C)
3. Test tube rack
4. Vortex Mixer

Kit Storage

The kit should be stored dry at room temperature for up to one month. For longer storage, the kit can be kept at 2–8°C for up to one year. Do not freeze the kit or any of its components.

Ordering Information

Cat. No.	Product	Quantity	Price
L00351	ToxinSensor™ Gel Clot Endotoxin Assay Kit	40 rxns	$90.00

Protocols

Quantitative Detection Protocols

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit

Proceed to the Reagent Preparation Area in a laminar flow cabinet at room temperature. Wear disposable gloves and use endotoxin-free materials in order to avoid contamination.

I **Specimen Preparation**

pH

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

II **Reagents Preparation**

Limulus Amebocyte Lysate (LAL)

Reconstitute lyophilized lysate by adding 1.7 ml LAL reagent water. Each reconstitution should be vortexed for 30 seconds with a vortex mixer or mixed gently by swirling. Do not shake or invert vortex to avoid foaming. Reconstituted lysate can remain stable if stored at -20°C for one week or for long-term use if frozen at -80°C immediately after reconstitution. Avoid repeated freeze and thaw cycles.

Chromogenic Substrate

Reconstitute the substrate by adding 1.7 ml of LAL reagent water to a concentration of ~2 mM. Once reconstituted, the substrate solution can remain stable for one month if stored at 2 - 8°C protected from light. Lyophilized chromogenic substrate can remain stable for one year if stored at 2 - 8°C.

Protocols, continued

Quantitative Detection Protocols, continued

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit, continued

Stop Solution

Reconstitute the color-stabilizer #1 (Stop Solution) with 10 ml of buffer S. Reconsituted Stop Solution can remain stable for one week if stored at 2 - 8°C.

Color-stabilizer #2 and #3

Reconstitute color-stabilizer #2 and 3 by adding 10 ml of LAL water for each. Each reconstitution can remain stable for one week at 2 - 8°C.

Standard endotoxin solutions

Dissolve 6 EU lyophilized endotoxin standard in 1 ml LAL reagent water to yield a concentration of 6 EU/ml. The dissolution should be vortexed for 15 minutes with a vortex mixer or mixed gently by swirling. Do not shake or invert vortex to avoid foaming. Store prepared endotoxin standard solutions (6 EU/ml) at -20°C for less than 24 hours. The solutions can remain stable for up to 15 days if frozen at -80°C . Dilute 0.1 ml of the 6 EU/ml endotoxin standard solution with 0.5 ml of LAL reagent water to make the 1 EU/ml standard solution. The 1 EU/ml standard solutions will be used for making the standard curve.

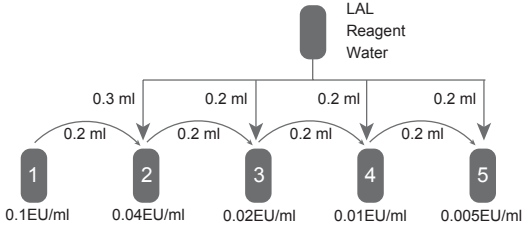
Protocols, continued

Quantitative Detection Protocols, continued

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit, continued

II **Reagents Preparation,** continued

In the assay example below, at least five serial dilutions of endotoxin solutions should be prepared to make a standard curve in each assay. If the expected endotoxin concentration range of samples is 0.005 - 0.1 EU/ml, the recommended concentrated endotoxin solutions should be 0.1, 0.04, 0.02, 0.01 and 0.005 EU/ml, respectively. The serial dilution of endotoxin solutions can be made as outlined in following figure. Each solution should be mixed thoroughly for 30 seconds with a vortex mixer.



Final Endotoxin Concentration

III **Test Procedure**

1. Carefully dispense 100 µl of standards, samples and LAL reagent water into different endotoxin-free vials and label them as standard 1, 2, 3, 4, 5, sample 1, 2, *etc*. and blank. Sample should be mixed thoroughly for 30 seconds with a vortex mixer too. Bubbles must be avoided.
2. Add 100 µl of reconstituted LAL to each vial. Cap the vials and vortexy for 3 seconds with a vortex mixer.
3. Incubate the rack with all vials in a 37°C oven for 45 minutes. If the endotoxin concentration is in the range of 0.1 - 1 EU/ml, incubate in a 37°C±1°C oven for only 10 minutes.

Protocols, continued

Quantitative Detection Protocols, continued

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit, continued

III **Test Procedure,** continued

4. After proper incubation, add 100 µl of reconstituted chromogenic substrate solution to each vial. Mix gently by swirling. Do not shake or invert vortex to avoid foaming, then incubate for 6 minutes in a 37°C oven.
5. Add 500 µl of reconstituted stop solution (color-stabilizer #1) to each vial and swirl gently to mix well. Do not shake or invert vortex to avoid foaming. Then add 500 µl of color-stabilizer #2 to each vial and mix well. Finally add 500 µl of reconstituted color-stabilizer #3 to each vial. Gently swirl each vial to mix well for 3 seconds. Bubbles must be avoided.
6. Read the absorbance of each reaction at 545 nm with distilled water as blank to adjust the photometer to zero absorbance.

The whole test procedure is also summarized in the following table:

	Standards	Samples	Blank
Standards (ml)	0.1	-	-
Samples (ml)	-	0.1	-
LAL Reagents Water (ml)	-	-	0.1
LAL (ml)	0.1	0.1	0.1
Mix well and incubate at 37°C + 1.0 °C (min)	45	45	45
Substrate solution (ml)	0.1	0.1	0.1
Mix well and incubate at 37°C + 1.0 °C (min)	6	6	6
Stop Solution (ml)	0.5	0.5	0.5
Color-stabilizer #2 (ml)	0.5	0.5	0.5
Color-stabilizer #3 (ml)	0.5	0.5	0.5
Mix well and read the absorbance at 545 nm			

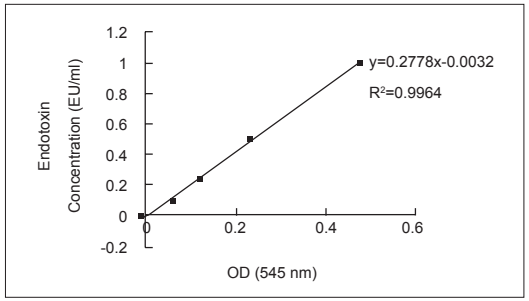
Protocols, continued

Quantitative Detection Protocols, continued

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit, continued

IV **Calculation Concentration**

Under the standard conditions, the absorbance at 545 nm is linear in the concentration range of 0.005 to 0.1 or 0.1 to 1 EU/ml endotoxin. Plot the mean absorbance for the four standards on the x-axis, the corresponding endotoxin concentration in EU/ml on the y-axis. Draw a best-fit line among these points and determine endotoxin concentrations of samples graphically.



If the mean absorbance value of a sample is x, the endotoxin concentration of the sample will be 0.2778x - 0.0032 EU/ml. All incubations were performed for 45 min.

Note: the OD values of standards may be differ in different endotoxin assays, the curve as above is only an example. The dilution of endotoxin standards and the incubation temperature are major factors that influence the OD value, so the endotoxin standards should be dissolved well in LAL water, and the incubation temperature should be 37±1°C.

Protocols, continued

Quantitative Detection Protocols, continued

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit, continued

V **Performance Characteristics**

Linearity

The linearity of the standard curve within the concentration range used to measure endotoxin values must be verified. At least 4 endotoxin standards spanning the expected concentration range should be assayed along with a blank, in duplicate. The absolute value of the coefficient of correlation (r) for the individual mean absorbance of the standards vs. their corresponding endotoxin concentration should be ≥0.980.

Reproducibility

Replicate samples should be run in order to establish good technique and low coefficient of variation. The coefficient of variation (C.V.) equals 100 times the standard deviation of a group of values divided by the mean and is expressed as a percent. The C.V. absorbance should be less than 10%.

Protocols, continued

Quantitative Detection Protocols, continued

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit, continued

VI **Troubleshooting**

Problem	Possible Cause	Suggestions
No linearity	Endotoxin standard is not mixed well.	Endotoxin may be adhering to glass surfaces. We recommend dissolving the 6 EU lyophilized endotoxin standard with 1 ml LAL reagent water , as stated by the protocol (the step 1 in "Test Procedure"), and mixing standard endotoxin dilutions for 15 minutes with a vortex mixer.
	The pH value of samples is not suitable for assay.	Adjust the pH value of your sample to pH 6-8, as stated by the protocol.
The negative blank shows a higher OD than that of standards using L00350.	The materials (e.g. tips, vials *etc.*) may be contaminated.	Proceed to the reagent preparation area in a laminar flow cabinet at room temperature. Wear disposable gloves and use endotoxin-free materials in order to avoid contamination.

Protocols, continued

Semi-Quantitative Detection Protocols

ToxinSensor™ Gel Clot Endotoxin Assay Kit

Proceed to the Reagent Preparation Area in a laminar flow cabinet at room temperature. Wear disposable gloves and use endotoxin-free materials in order to avoid contamination.

I **Specimen Preparation**

pH

The pH value of the sample should be at pH 6-8 to ensure good linearity. Consequently, we recommend adjusting the PH value with HCl or NaOH.

Dilution

Dilution is the most important strategy for dealing with interference. Consequently, samples should be diluted with LAL reagent water before proceeding. In addition, it is necessary to calculate the MVD* to ensure a margin of safety, we recommend not exceeding the MVD of your sample.

*** MVD** (Maximum Valid Dilutionis): a dilution factor showing endotoxin limit (in EU/ml) divided by lambda. The labeled lysate reagent sensitivity in the gel-clot methods of our kit is 0.25 EU/ml.

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

Protocols, continued

Semi-Quantitative Detection Protocols, continued

ToxinSensor™ Gel Clot Endotoxin Assay Kit, continued

II **Reagent preparation**

Limulus Amebocyte Lysate (LAL)

Reconstitute lyophilized lysate by adding 2 ml LAL reagent water before proceeding. Each reconstitution should be vortexed for 30-60 seconds with a vortex mixer or mixed gently by swirling. Do not shake or invert vortex to avoid foaming. Reconstituted lysate can remain stable for one week if stored at -20°C, or for long-term use if frozen at -80°C immediately after reconstitution. Avoid repeated freeze and thaw cycles.

Positive controls

Reconstitute *E. coli* endotoxin Standard by adding 1 ml LAL reagent water to a concentration of 0.5 EU/ml. The reconstitution should be vortexed for at least 15 minutes with a vortex mixer. Reconstituted *E. coli* endotoxin standard can remain stable for up to 15 days if stored at -20°C.

III **Test procedure**

Appropriate positive and negative controls are an integral part of each assay. LAL Reagent Water can be used as a negative control.

1. Carefully dispense 0.1 ml of LAL solution into different Endotoxin-free vials. Label them negative control, positive control, and samples.
2. Carefully transfer 0.1 ml of positive control, negative control and the test samples to the LAL reagent in prepared dispensing vials. Cap the vials and mix them thoroughly.

Protocols, continued

Semi-Quantitative Detection Protocols, continued

ToxinSensor™ Gel Clot Endotoxin Assay Kit, continued

III **Test procedure,** continued

3. Incubate the incubation rack with all vials in 37°C ±1°C with non-circulating hot water or oven. Keep racks standing while incubating.
4. 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 gel consistency.
   a) A positive reaction is characterized by the formation of a firm gel that remains intact when the vial is inverted.
   b) A negative reaction is characterized by the absence of a solid clot. The lysate may show an increased turbidity or viscosity. This is considered a negative result.
5. Calculate of endotoxin level. In this test, the endotoxin level in the positive sample is equal or higher than 0.25 EU/ml, while in the negative sample is lower than 0.25.

IV **Example**

1. Sample: 1 mg/ml Protein A provided in PBS (pH 7.4). The Protein A is purified from *E. coli* sonicate by Ni-NTA Resin.
2. Prepare dilution in LAL reagent water according to following dilution times: 1: 200,000, 1: 400,000, 1: 800,000

Protocols, continued

Semi-Quantitative Detection Protocols, continued

ToxinSensor™ Gel Clot Endotoxin Assay Kit, continued

IV **Example,** continued

3. The test is performed as above protocol states, and the assay result is:

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

4. Endotoxin concentration value* of this sample is in the range of 50,000 - 100,000 EU/ml

*Endotoxin Concentration Value = Dilution Times × 0.25 EU/ml

V **Troubleshooting**

Problem	Possible Cause	Suggestions
Negative control produces a gel using L00351.	The materials (e.g. tips, vials etc.) may be contaminated.	Pay more attention to operation and keep the assay under laminar flow cabinets.
Positive control does not form gel.	The standard of endotoxin is not mixed well.	The standard should be vigorously vortexed for 15 minutes prior to use.

Customer References

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PMID 19751832

Related Products

Cat. No.	Products	Quantity	Price
M01062	ToxinSensor™ Endotoxin-free Vials (2 ml, Clear)	16 Vials	$20.00
M01063	ToxinSensor™ Endotoxin-free Pipette Tips (1 ml, Blue)	6 Tips	$6.00
L00338	ToxinEraser™ Endotoxin Removal Kit	3 - 5 Assays	$110.00
L00408	ToxinEraser™ Endotoxin Removal Advanced Kit	3 - 5 Assays	$220.00
L00402	ToxinEraser™ Endotoxin Removal Resin	1 ml	$60.00
M01053	ToxinEraser™ Regeneration Buffer	125 ml	$25.00
M01054	ToxinEraser™ Equilibration Buffer	125 ml	$25.00

Technical Support

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