ToxinSensor™

Endotoxin Detection System

Version 07072023

User Manual

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Intended Use

GenScript ToxinSensor™ Endotoxin Detection System is an *in vitro* end-point endotoxin test system for human and animal parenteral drugs, biological products, and medical devices. The system is not intended for detection of endotoxin in a licensed reagent, clinical samples or the diagnosis of human disease. For end-point chromogenic method(Cat. No.: L00350 & L00350C), a measurable endotoxin concentration range from 0.01 to 1 EU/mL can be achieved. For the gel method(Cat. No.: L00351), the sensitivity is 0.25 EU/mL. LAL (Lyophilized Amebocyte Lysate) reagent is made from amebocyte lysate from the horseshoe crab (*Tachypleus tridentatus*). These kits can be used according to the bacterial endotoxin test method of the US Pharmacopoeia and the Chinese Pharmacopoeia.

Warning

For specimen preparation, the specimen should be certified free of Beta Glucans contaminant. Not for endotoxemia in human or animals, or clinical diagnosis, patient management, cell / bacterial culture medium, serum, blood or blood products.

Background

For biopharmaceutical companies, endotoxin detection is acritical quality control test to ensure that manufacturing of pharmaceutical products are free of endotoxin contaminations. There are three common methods, below is a general selection guide of choosing which method to use:

Methods	Maximum Sensitivity	Additional Material Required
Gel-clot	0.03 EU/mL	Non-circulating water bath or dry bath incubator
Chromogenic	0.01 EU/mL	A microplate reader (an incubating reader is required for the kinetic method)
Turbidimetric	0.001 EU/mL	An incubating microplate reader

Ordering Information

Cat. No.	Size	Test	Methods	Sensitivity
L00350C	16 rxns	Quantitative	Endpoint	0.01 to 1 EU/mL
L00350	32 rxns		Chromogenic	
L00351	40 rxns	Semi-quantitative	Gel-clot	0.25 EU/mL

Protocol: ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat.No.: L00350&L00350C)

ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit

This kit is designed as a quantitative assay 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 spectrophotometrically and compared to a standard curve. A measurable endotoxin concentration range from 0.01 to 1 EU/mL can be achieved.

Features

- Good linearity and good reproducibility
- High sensitivity and board application range
- Ready-to-use reagents and materials

Kit Contents

PK				Label	Volume
Product: L00350		Product: L00350C			
L00350	L00350Y	L00350C	L00350CY		
3 bottles	-	2 bottles	-	LAL Reagent Water	50 mL
-	2 vials	-	1 vial	LAL	-
-	2 vials	-	1 vial	*E. coli* Endotoxin Standard	-
-	2 vials	-	1 vial	Chromogenic Substrate	-
2 vials	-	1 vial	-	Color-stabilizer #1	-
2 vials	-	1 vial	-	Color-stabilizer #2	-
2 vials	-	1 vial	-	Color-stabilizer #3	-
10 × 5 vials	-	5 × 5 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	-

Protocol: ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat.No.: L00350&L00350C)

Materials and equipments not provided
1. Concentrated hydrochloric acid, 36-38%
2. Sodium hydroxide, 0.1M, dissolved in LAL Reagent Water. The reagent is for adjustment of the pH of samples if necessary
3. Hydrochloric acid, 0.1M, dissolved in LAL Reagent Water. The reagent is for adjustment of the pH of samples if necessary
4. Parafilm
5. Water bath or heating blocks set at 37 ± 1.0°C
6. Spectrometer or filter photometer with a 545 nm filter
7. Vortex mixer
8. Timer

Package Storage

Please store L00350Y, L00350CY at 2-8°C and store L00350, L00350C at cool and dark place. Do NOT freeze the kit or any of its components.

The COA file can be obtained from the official website of GenScript through Cat. No. and Lot. No.

Quantitative Detection Protocols

A. Specimen Preparation

All materials or diluents used for specimen collection and preparation or test reagents must be endotoxin-free. Use aseptic technique at all times. Sample to be tested must be stored in a way that all bacteriological activities are blocked. For example, samples can be stored at 2-8°C within 24 hours before using, but need to be frozen for long-term use. If the sample has high endotoxin level, it is recommended to vortex properly before testing to prevent false negatives due to endotoxin accumulation.

Protocol: ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat.No.: L00350&L00350C)

Quantitative Detection Protocols

Test For Interfering Factors

The varieties without endotoxin inspection items should be tested for interference in accordance with the content of bacterial endotoxin detection in the Pharmacopoeia when this inspection method is newly established. If there are any changes that may affect the experimental results, the interference test needs to be repeated.

pH

Dissolve or dilute test specimen using LAL Reagent Water. Since the LAL-endotoxin reaction is pH dependent, the pH value of the sample should be at pH 6-8 (18-26°C) to ensure good linearity. Consequently, we recommed adjusting pH value using sodium hydroxide (0.1M, dissolved in LAL Reagent Water) or hydrochloric acid (0.1 M, diluted in LAL Reagent Water) if necessary.

MVD

Maximum valid dilution is calculated by dividing the endotoxin limit (in EU/mL) by λ. For the end-point chromogenic method kit, λ is the lower limit of the selected curve range. For example, when the curve of 0.01-0.1 EU/mL is selected for the experiment, the sensitivity λ=0.01 EU/mL. When the estimated endotoxin level of the sample is outside the detection range, the sample needs to be diluted before detection. The dilution factor is determined by the MVD. For reliable results, do not exceed the MVD of the sample.

Protocol: ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat.No.: L00350&L00350C)

Quantitative Detection Protocols

B. Reagents Preparation

Note: It is recommended to dissolve the LAL powder before adding to the test tube. Other reagents can be prepared in advance.

***E. coli* Endotoxin Standard**

The amount of lyophilized endotoxin standard supplied is mentioned on the label on the vial of the *E. coli* Endotoxin Standard in the kit.

Dissolution： It is recommended to use an appropriate volume of LAL Reagent water to reconstitute the endotoxin standard. Mix thoroughly for 15 minutes by vortexing to obtain an endotoxin stock solution。

If the titer of endotoxin standard P>50 EU/vial, (P/50) mL of LAL reagent water can be added to prepare a concentration of 50 EU/mL of endotoxin standard substance solution, and then diluted gradually according to the needs of the experiment.

If the titer of endotoxin standard 20＜P≤50 EU/ vial, (P/20) mL of LAL reagent water can be added to prepare a concentration of 20 EU/mL of endotoxin standard substance solution, and then diluted gradually according to the needs of the experiment.

If the titer of endotoxin standard P≤20 EU/vial, (P/10) mL of LAL reagent water can be added to prepare a concentration of 10 EU/mL of endotoxin standard substance solution, and then diluted gradually according to the needs of the experiment.

Protocol: ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat.No.: L00350&L00350C)

Quantitative Detection Protocols

Dilution: These endotoxin standard solutions can be further diluted with LAL reagent water into different concentration gradients. The dilution should be mixed thoroughly for 30 seconds with a vortex. Each dilution should not exceed 10 times.

Note: The endotoxin stock solution needs to be vortexed for at least 1 minute if stored for more than 10 minutes. Endotoxin standard solution that has been stored for more than 4 hours should be discarded. Freezing endotoxin standard solution is not recommended.

LAL

Reconstitute LAL by adding 1.7 ml LAL Reagent Water. Gently invert to dissolve the lyophilized powder, then wait 20-30 seconds for the liquid to clear. Avoid foaming during the process. Do not use violent method to dissolve LAL, such as pipetting, vortexing, heating and ultrasound , which may affect its stability and sensitivity.

Note: LAL Reagent must be used up within 10 minutes. The solution was freshly prepared just before use.

Protocol: ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Cat.No.: L00350&L00350C)

Quantitative Detection Protocols

Chromogenic Substrate

Reconstitute the substrate by adding 1.7 mL of LAL Reagent Water. Once reconstituted, the substrate solution can remain stable for one month if stored at 2-8°C. Protect substrate solution from long direct exposure to light.

Color-stabilizer #1

Firstly, add 2 mL concentrated hydrochloric acid to 50 mL LAL Reagent Water with sufficient mixing and make a final concentration of 0.46 M HCl.

Note: This step should be carried out in a hood because of the escape of poisonous hydrochloric acid.
Then, reconstitute the color-stabilizer#1with 10mL of 0.46 M HCl, the solution also called Stop Solution. It can be stable for one week when stored at 2-8°C.

Color-stabilizer #2、#3

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

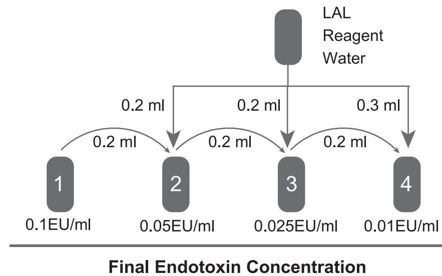
Preparation of standard solutions of different concentrations

Prepare 1 EU/mL endotoxin solution to make standard serial dilutions. In each assay, at least 3 endotoxin standard solutions covering desired concentration range should be prepared to generate a standard curve. E.g. if the endotoxin concentration for the test sample is expected to be in the range of 0.01-0.1 EU/mL, the serial endotoxin standard solutions could be 0.1, 0.05, 0.025 and 0.01 EU/mL, respectively. If the endotoxin concentration

Protocol: ToxinSensorTM Chromogenic LAL Endotoxin Assay Kit (Cat.No.: L00350&L00350C)

Quantitative Detection Protocols

for the test sample is expected to be in the range of 0.1-1.0 EU/mL, the serial endotoxin standard solutions could be 1.0 , 0.5, 0.25 and 0.1 EU/mL, respectively. An example of the preparation of serial endotoxin standard solutions is outlined in the figure below. Each solution should be mixed thoroughly for at least 30 seconds by vortex. Make sure to seal the tubes with parafilm during dilution so that no liquid spills.



Final Endotoxin Concentration

C. Test Procedure

Note: All reagent dosage and reaction time cannot be adjusted. When incubating, be sure to cover all the tubes with tin foil wrapping the tubes. Sealing one by one will prolong the incubation time.

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, sample 1, 2, etc. and blank. Sample should be mixed thoroughly for 30 seconds with a vortex mixer too. Avoid foaming.

2. Add 100 μL of reconstituted LAL to each vial and mix well by swirling gently.

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3. If the expected endotoxin concentration range of samples is 0.01-0.1 EU/mL, incubate the rack with all vials at 37±1°C using water bath or heating blocks for T1. If the endotoxin concentration is in the range of 0.1-1 EU/mL incubate in a 37±1°C using water bath or heating blocks for T2. The optimal value of T1 and T2 should be referred to the label on the kit.

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±1°C using water bath or heating blocks inmidiately.

5.Then 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 mix well. Avoid foaming.

6. Read the absorbance of each reaction at 545 nm. Use distilled water as blank to adjust the photometer to zero absorbance. The result can also be read by Microplate Reader.
Transfer 200 μL of the final solution into a 96-well plate to read the absorbance at 545 nm. If the reading cannot be done immediately, seal the test solution and ensure that it is completed within 5 hours.

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Quantitative Detection Protocols

The whole test procedure is summarized in the table below:

	Standards	Samples	Blank
Standards (mL)	0.1	-	-
Samples (mL)	-	0.1	-
LAL Reagent Water (mL)	-	-	0.1
LAL (mL)	0.1	0.1	0.1
Mix well and incubate at 37 ± 1.0°C	T1 or T2	T1 or T2	T1 or T2
Substrate solution (mL)	0.1	0.1	0.1
Mix well and incubate at 37 ± 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			

D. Calculation of Concentration

Under the standard conditions, the absorbance at 545 nm shows a linear relationship with the concentration in range of both 0.01-0.1 EU/mL and 0.1-1 EU/mL. Plot the blank-subtracted absorbance of the four standards on the y-axis, the corresponding endotoxin concentration in EU/ml on the x-axis. Draw a best-fit straight line among these points and calculate endotoxin concentrations of samples graphically.

Examples used data below:

Tube No.	Sample	Absorbance at 545nm	Δ Absorbance
1	LAL Reagent Water (Blank)	0.0525	-
2	0.01 EU/ml Standard	0.0965	0.0441
3	0.025 EU/ml Standard	0.1650	0.1125
4	0.05 EU/ml Standard	0.3139	0.2614
5	0.1 EU/ml Standard	0.6702	0.6177

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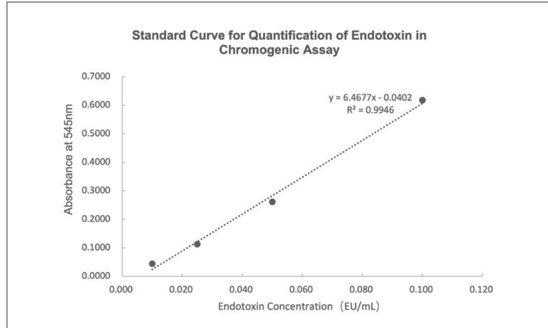
Quantitative Detection Protocols



If the average absorbance of the sample is y, the relationship between the endotoxin concentration (x) of the sample and the average absorbance of the sample is y = 6.4677x-0.0402. Figure above is only an example curve, the OD values of standards may be different with different assays.

Note: Standard dissolution, dilution accuracy, incubation temperature, and incubation time are key factors affecting the results. It must be ensured that the endotoxin standard is completely dissolved and accurately diluted. The incubation temperature should be strictly maintained at 37 ± 1°C, and the addition time should be shorten as much as possible.

E. Performance Characteristics Linearity

Linearity

The linearity of the standard curve within the concentration range used to measure endotoxin values must be verified. At least 3 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.

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Quantitative Detection Protocols

Reproducibility

Replicate samples should be used 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%.

Troubleshooting

Problem	Possible Cause	Suggestions
No linearity	Insufficient endotoxin vortexing	Endotoxin may adhere to the surface of glass and may form aggregates. It is generally recommended to add an appropriate amount of LAL reagent water to dissolve. Place it on a vortex mixer for at least 15 minutes, and it is recommended to use automatic vortex equipment for vorticity.
	Overreacting or underreacting	The result of overreaction is that absorbances of 1EU/mL and 0.5EU/mL are all close to the limit. It is recommended to strictly control the time introduced by the sample addition process. Insufficient reaction results in very low absorbance for all solutions. And pay attention to use a water bath for incubation as much as possible.
The blank shows a higher OD than standard solutions	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.

Protocol: ToxinSensor™ Gel Clot Endotoxin Assay Kit

(Cat.No.: L00351)

ToxinSensor™ Gel Clot Endotoxin Assay Kit

This kit is designed to be the simplest semi-quantitative test for gram-negative bacterial endotoxin. The LAL provided in the kit should be reconstituted with LAL reagent water, then mixed with an aliquot of the solution to be tested and incubated at 37°C for the appropriate time. Gels form in the presence of endotoxin; no gelation occurs in the absence of endotoxin.

Features

- Good reproducibility
- Board application range
- Ready-to-use reagents and materials

Kit Contents

PK	Label	Volume
2 bottles	LAL Reagent Water	50 mL
2 vials	LAL	2.2 mL
2 vials	*E. coli* Endotoxin Standard	-
17 × 5 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	-

Protocol: ToxinSensor™ Gel Clot Endotoxin Assay Kit

(Cat.No.: L00351)

Materials and equipments not provided
1. Sodium hydroxide, 0.1M, dissolved in LAL Reagent Water. The reagent is for adjustment of the pH of samples if necessary
2. Hydrochloric acid, 0.1M, dissolved in LAL Reagent Water. The reagent is for adjustment of the pH of samples if necessary
3. Parafilm
4. Water bath or heating blocks set at 37 ± 1.0°C
5. Vortex mixer
6. Timer

Package Storage

The kit can be stored at room temperature for up to one month. Please store at 2-8°C for longer storage. Do **NOT** freeze the kit or any of its components.

Semi-Quantitative Detection Protocols

A. Specimen Preparation

All materials or diluents used for specimen collection and preparation or test reagents must be endotoxin-free. Use aseptic technique at all times. Sample to be tested must be stored in a way that all bacteriological activities are blocked. For example, samples can be stored at 2-8°C within 24 hours before using, but need to be frozen for long-term use. If the sample has high endotoxin level, it is recommended to vortex properly before testing to prevent false negatives due to endotoxin accumulation.

Test For Interfering Factors

The varieties without endotoxin inspection items should be tested for interference in accordance with the content of bacterial endotoxin detection in the Pharmacopoeia when this inspection method is newly established. If there are any changes that may affect the experimental results, the interference test needs to be repeated.

Protocol: ToxinSensor™ Gel Clot Endotoxin Assay Kit

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pH

Dissolve or dilute test specimen using LAL Reagent Water. Since the LAL-endotoxin reaction is pH dependent, the pH value of the sample should be at pH 6-8 (18-26°C) to ensure good linearity. Consequently, we recommed adjusting pH value using sodium hydroxide (0.1M, dissolved in LAL Reagent Water) or hydrochloric acid (0.1 M, diluted in LAL Reagent Water) if necessary.

MVD

Maximum valid dilution is calculated by dividing the endotoxin limit (in EU/mL) by λ. The labeled LAL reagent sensitivity of kit L00351 is 0.25 EU/mL. When the estimated endotoxin level of the sample is outside the detection range, the sample needs to be diluted before detection. The dilution factor is determined by the MVD. For reliable results, do not exceed the MVD of the sample.

B. Reagents Preparation

Note: It is recommended to dissolve the LAL powder before adding to the test tube.

***E. coli* Endotoxin Standard**

The titer of lyophilized endotoxin standard supplied is mentioned on the label on the vial of the *E. coli* Endotoxin Standard in the kit.

Dissolution： It is recommended to use an appropriate volume of LAL Reagent water to reconstitute the endotoxin standard. Mix thoroughly for 15 minutes by vortexing to obtain an endotoxin stock solution。

If the titer of endotoxin standard P > 50 EU/vial, (P/50) mL

Protocol: ToxinSensor™ Gel Clot Endotoxin Assay Kit

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Semi-Quantitative Detection Protocols

of LAL reagent water can be added to prepare a concentration of 50 EU/mL of endotoxin standard substance solution, and then diluted gradually according to the needs of the experiment.

If the titer of endotoxin standard 20＜P≤50 EU/ vial, (P/20) mL of LAL reagent water can be added to prepare a concentration of 20 EU/mL of endotoxin standard substance solution, and then diluted gradually according to the needs of the experiment.

If the titer of endotoxin standard P≤20 EU/vial, (P/10) mL of LAL reagent water can be added to prepare a concentration of 10 EU/mL of endotoxin standard substance solution, and then diluted gradually according to the needs of the experiment.

Dilution: These endotoxin standard solutions can be further diluted with LAL reagent water into different concentration gradients. The dilution should be mixed thoroughly for 30 seconds with a vortex. Each dilution should not exceed 10 times.

Note: The endotoxin stock solution needs to be vortexed for at least 1 minute if stored for more than 10 minutes. Endotoxin standard solution that has been stored for more than 4 hours should be discarded. Freezing endotoxin standard solution is not recommended.

LAL

Reconstitute LAL by adding 2.2 mL LAL Reagent Water. Gently invert to dissolve the lyophilized powder, then wait 20-30 seconds for the liquid to clear. Avoid foaming during the process. Reconstituted LAL can remain stable for one

Protocol: ToxinSensor™ Gel Clot Endotoxin Assay Kit

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Semi-Quantitative Detection Protocols

week if stored at -20°C under aseptic condition, longer storage is not recommended. Avoid repeated freeze and thaw cycles. Do not use violent method to dissolve LAL, such as pipetting, vortexing, heating and ultrasound , which may affect its stability and sensitivity.

Note: LAL Reagent must be used up or stored within 10 minutes.

Test For Confirmation Of Labeled LAL Sensitivity

The test for confirmation of lysate sensitivity is to be carried out when a new batch of lysate is used or when there is any change in the test conditions that may affect the outcome of the test. If this is not less than 0.5λ and not more than 2λ, the labeled sensitivity is confirmed and is used in tests performed with this lysate. Please refer to the relevant content of the Pharmacopoeia for specific experimental procedures. It should be noted that the endotoxin standard used should be batch-matched CSE (the *E. coli* Endotoxin Standard provided in the kit) or RSE.

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Semi-Quantitative Detection Protocols

Positive control

Prepare 0.5 EU/mL endotoxin solution as a positive control. For example, if the endotoxin stock solution is 20 EU/mL, dilute it with LAL Reagent Water to make the 0.5 EU/mL solution. The dilution should be mixed thoroughly for 30 seconds with a vortex. Each dilution should not exceed 10 times.

Negative control

LAL reagent water can be used as a negative control.

Positive control for test sample

Test sample containing 0.5 EU/ml endotoxin standard solution.

Test sample

Sample to be tested.

C. Test Procedure

Note: Typically, at least 2 parallel sets of each reaction are required to ensure reliable results.

1. Carefully open the foil packaging on the outside of the endotoxin-free tubes, avoid microbial and endotoxin contamination. If using a water bath for incubation, the foil packaging can be used to cover the tubes.
2. Carefully dispense 0.1 mL of LAL reagent into different endotoxin-free tubes.

Protocol: ToxinSensor™ Gel Clot Endotoxin Assay Kit

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Semi-Quantitative Detection Protocols

3. Carefully transfer 0.1 mL of positive control, negative control, test article positive control, and all test samples to the LAL in prepared dispensing vials Step2 and mix by gently shaking 3-5 times.

4. Incubate all tubes in a water bath or a endotoxin gel heater at 37 ±1°C. Keep the tubes upright and avoid shaking while incubating.

5. After 60 minutes of incubation, invert each tube gently and check for gel formation.

6. Do not shake vigorously or invert multiple times during inspection, which may destroy the gel state.
   a) A positive reaction is characterized by the formation of a firm gel that remains intact when thevial 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 anegative result.

7. The experiment is valid only when the negative control is not gelled and the positive control and the positive control of the test sample are gelled.
   a) If the negative control gels, it may be caused by contamination from the environment or the consumables used.
   b) If the positive control does not gel, it may be due to failure of the LAL reagent, reduced titer of the endotoxin standard, insufficient dissolution of standard or inadequate incubation equipment.

Protocol: ToxinSensor™ Gel Clot Endotoxin Assay Kit

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Semi-Quantitative Detection Protocols

c) If the positive control of the test sample does not gel, there may be interference in the system.

8. Determination of endotoxin level: In this test, a series of dilutions of the test sample can be tested at the same time. When the result is positive, it indicates that the endotoxin level of the tube is equal to or higher than 0.25 EU/mL. When the result is negative, it indicates that the endotoxin level of the tube is lower than 0.25 EU/mL. The original endotoxin level needs to be multiplied by the corresponding dilution factor.

D. Example Preparation

1. Sample: 1 mg/mL Protein A provided in PBS (pH 7.4). The Protein A is purified from *E. coli* sonicate by High Affinity Ni-Charged Resin. There is no interference factor in this sample, and no positive control for the test sample is set.

2. Prepare dilution in LAL Reagent Water according to following dilution times: 1: 200,000, 1: 400,000, 1: 800,000.

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

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

4. Endotoxin concentration value* of this sample ranges from 50,000 to 100,000 EU/ml

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

Protocol: ToxinSensor™ Gel Clot Endotoxin Assay Kit

(Cat.No.: L00351)

Semi-Quantitative Detection Protocols

E. Troubleshooting

Problem	Possible Cause	Suggestions
Negative control produces a gel	The materials (e.g. tips, vials *etc.*) may be contaminated.	Pay more attention to operation and keep the assay in a laminar flow cabinet.
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. Automatic vortexing equipment is recommended.
	The endotoxin standard does not match the LAL batch.	Use the standard in the same kit.
	The potency of endotoxin standard decreased for incorrect storage conditions or frequent freezing and thrawing.	Prepare a new matched batch of endotoxin standard.

Selected Citations

Shalaby KH., et al. Inhaled Birch Pollen Extract Induces Airway Hyperresponsiveness via Oxidative Stress but Independently of Pollen-Intrinsic NADPH Oxidase Activity, or the TLR4-TRIF Pathway. J Immunol. **2013** Jul 15;191(2): 922-33.

Clark KD., et al. Hemolymph melanization in the silkmoth Bombyx mori involves formation of a high molecular mass complex that metabolizes tyrosine. J Biol Chem. **2013** May 17;288(20): 14476-87.

Kim EH., et al. Prokaryote-expressed M2e protein improves H9N2 influenza vaccine efficacy and protection against lethal influenza a virus in mice. Virol J. **2013** Apr 3;10 : 104.

O'Meara CP., et al. Immunization with a MOMP-Based Vaccine Protects Mice against a Pulmonary Chlamydia Challenge and Identifies a Disconnection between Infection and Pathology. PLoS One.**2013** Apr 16;8(4): e61962.

Kaliannan K., et al. Intestinal alkaline phosphatase prevents metabolic syndrome in mice. Proc Natl Acad Sci USA. **2013** Apr 23;110(17): 7003-8.

Gomez G., et al. Immunogenic and Invasive Properties of Brucella melitensis 16M Outer Membrane Protein Vaccine Candidates Identified via a Reverse Vaccinology Approach. PLoS One. **2013**;8(3): e59751.

Chen H., et al. In Vivo Study of Spherical Gold Nanoparticles: Inflammatory Effects and Distribution in Mice. PLoS One. 2013;8(2): e58208.

Related Products

Cat. No.	Products	Quantity
L00338	ToxinEraser™ Endotoxin Removal Kit	1 kit
L00402	ToxinEraser™ Endotoxin Removal Resin	1 ml
M01053	ToxinEraser™ Regeneration Buffer	125 ml
M01054	ToxinEraser™ Equilibration Buffer	125 ml
M01063	ToxinSensor™ Endotoxin-free Pipette Tips (1 ml, Blue)	1PK of 6 Tips
M01072-5	ToxinSensor™ Endotoxin-free Tubes	5 Tubes
M01072-10	ToxinSensor™ Endotoxin-free Tubes	10 Tubes
M01072-40	ToxinSensor™ Endotoxin-free Tubes	40 Tubes
L00856-20	ToxinSensor™ Single Tests Kit with Standard, 0.03EU/ml	20 assay
L00856-40	ToxinSensor™ Single Tests Kit with Standard, 0.03EU/ml	40 assay
L00857-20	ToxinSensor™ Single Tests Kit with Standard, 0.06EU/ml	20 assay
L00857-40	ToxinSensor™ Single Tests Kit with Standard, 0.06EU/ml	40 assay
L00858-20	ToxinSensor™ Single Tests Kit with Standard, 0.125EU/ml	20 assay
L00858-20	ToxinSensor™ Single Tests Kit with Standard, 0.125EU/ml	40 assay
L00859-20	ToxinSensor™ Single Tests Kit with Standard, 0.25EU/ml	20 assay
L00859-40	ToxinSensor™ Single Tests Kit with Standard, 0.25EU/ml	40 assay

Technical Support

Visit the GenScript Web site at www.genscript.com for:

1. Technical resoures, including manuals, MSDS, FAQ, etc.

2. Online Product Catalog

3. Additional promotions and special offers

Any question about Products, please email us at product@genscript.com

生产商：南京金斯瑞生物科技有限公司 江苏省南京市江宁区科学园雍熙路28号

Manufacturer: Nanjing GenScript Biotech Co., Ltd. No. 28 Yongxi Road, Jiangning District, Nanjing, Jiangsu, China

GenScript USA Inc.

860 Centennial Ave.

Piscataway, NJ 08854

Toll-Free: 877-436-7274

Tel: 732-885-9188, 732-885-9688

Fax: 732-210-0262, 732-885-5878

Email: product@genscript.com

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