Datasheet for ABIN491526

ToxinSensor Chromogenic LAL Endotoxin Assay Kit

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Overview

Quantity:	32 tests
Reactivity:	Human, Various Species
Detection Range:	0.005-1 U/mL
Minimum Detection Limit:	0.005 U/mL
Application:	Cytotoxicity Test (CyTox)

Product Details

Brand:	ToxinSensor™
Characteristics:	ToxinSensor ™ Chromogenic LAL Endotoxin Assay Kit is designed to be used as a quantitative In Vitro end-point endotoxin test for human and animal parenteral drugs, biological products, and medical devices. This method utilizes a modified Limulus Amebocyte Lysate and a synthetic color producing substrate to detect endotoxin chromogenically. The kit has a minimum endotoxin detection limit of 0.005 EU/mL and a measurable concentration range of 0.005 to 1 EU/mL. In addition, any sample with color (e.g. bacterial/cell culture medium, serum or blood etc.) cannot be assayed using this kit.
Components:	LAL Reagent Water, 50 mL/bottle (2 bottles) Limulus Amebocyte Lysate (LAL) (2 Vials) E. coli Endotoxin Standard (2 Vials) Chromogenic Substrate 1 Vial 2 Vials Buffer S for Color-stabilizer #1 (50 mL) Color-stabilizer #1 (2 Vials) Color-stabilizer #2 (2 Vials) Color-stabilizer #3 (2 Vials)

Product Details

	Endotoxin-free tubes (50) Endotoxin-free Tips, 200 µL (1 box (96 tips)) Endotoxin-free Tips, 1000 µL (2 bags (12 tips)) Incubation Rack (1)
Material not included:	1. Sodium hydroxide, 0.1 N, dissolved in LAL Reagent Water, for pH adjustment. 2. Hydrochloric acid, 0.1 N, diluted in LAL Reagent Water, for pH adjustment. 3. Water bath or heating block set at 37°C. 4. Spectrometer or filter photometer with a 545 nm filter. 5. Vortexer. 6. Timer.

Target Details

Background:	Genscript ToxinSensorTM Chromogenic LAL Endotoxin Assay Kit is designed to be a quantitative In Vitro end-point endotoxin test for human and animal parenteral drugs, biological products, and medical devices. This method utilizes a modified Limulus Amebocyte Lysate and a synthetic color producing substrate to detect endotoxin chromogenically in a broad range of 0.005 - 1 EU/mL. In addition, any sample with color (e.g. cell bacterial culture medium, serum or blood etc.) can not be assayed by this kit.

Application Details

Comment:	Note: The dilution of standards and incubation temperature would be critical factors that can influence the absorption value, so it?s important to make sure that the endotoxin standard is fully dissolved, and the incubation temperature should strictly be kept at 37 °C.
Reagent Preparation:	Limulus Amebocyte Lysate (LAL) Reconstitute lyophilized lysate by adding 1.7 mL LAL Reagent Water. Swirl each reconstitution gently for 30 seconds, avoid foaming. Reconstituted lysate remains stable if stored at -20°C for one week. For long-term usage, freeze the lysate 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 final concentration of ~2 mM. Once reconstituted, the substrate solution is stable for one month when stored at 2°C - 8°C. PROTECT SUBSTRATE FROM LONG-TERM EXPOSURE TO LIGHT Stop Solution Reconstitute the Color-stabilizer #1 (Stop Solution) with 10 mL of buffer S. The reconstituted

	Stop Solution is stable for one week when stored at 2°C - 8°C. Color-stabilizer #2 and #3 Reconstitute Color-stabilizer #2 and #3 by adding 10 mL of LAL Reagent Water for both, respectively. Each reconstitution is stable for one week when stored at 2°C - 8°C. Endotoxin Standard Solution The amount of lyophilized endotoxin standard supplied in this kit is approximately 5 - 20EU. Please refer to the label on the vial of the endotoxin standard in the kit. Dissolve lyophilized endotoxin standard by adding 2 mL of LAL Reagent Water. Mix thoroughly for 15 minutes with a vortexer to obtain an endotoxin stock solution. The reconstituted endotoxin stock solution is stable for one week when stored at 2-8°C. DO NOT FREEZE THE ENDOTOXIN STOCK SOLUTION. Prepare 1EU/mL endotoxin solution before making standard serial dilutions. For example, if the endotoxin stock solution is 5 EU/mL, dilute 0.2 mL of 5 EU/mL endotoxin stock solution with 0.8 mL of LAL Reagent Water to make the 1 EU/mL solution. In each assay, at least four endotoxin standard solutions covering desired concentration range should be prepared to generate a standard curve. 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 in sample is expected in the range of 0.1 - 1 EU/mL, the serial endotoxin standard solutions could be 1, 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 30 seconds with a vortexer.
Sample Preparation:	All materials or diluents used for specimen collection and test reagent preparation must be endotoxin-free. Use aseptic technique at all times. Samples to be tested must be stored in such a way that all bacteriological activity is stopped or the endotoxin level may increase over time. For example, samples can be stored at 2-8 °Cwithin 24 hours before use, but need to be stored frozen if not used within 24 hours. Dissolve or dilute test specimen using LAL Reagent Water. Since the LAL-endotoxin reaction is pH dependent, the pH value of the mixture should be between 6.0 and 8.0 to ensure standard and data linearity. Adjust the pH of the specimen with endotoxin-free 0.1N sodium hydroxide or 0.1N hydrochloric acid. Always measure the pH of an aliquot of the bulk sample, take care to avoid contamination by the pH electrode used. If the specimen contains interfering substances, dilute the specimen until that the interference is eliminated.
Assay Procedure:	1) Carefully dispense 100 µL of standard or test sample into endotoxin-free vials. All samples should be mixed thoroughly for 30 seconds with a vortexer. Avoid foaming/bubbles. Each test must include a blank as well as at least four endotoxin standards in duplicate. The blank

	sample vial contains 100 µL of LAL Reagent Water instead of test sample. 2) Add 100 µL of reconstituted LAL to each vial. Cap the vials and mix well by swirling gently. 3) If the endotoxin concentration in sample is expected in the range of 0.01 - 0.1 EU/mL, incubate the rack with all vials at 37°C±1°C for 45 minutes using a water bath or heating block. If the endotoxin concentration is expected in the range of 0.1 - 1 EU/mL, incubate at 37°C±1°C for 10 minutes. 4) After proper incubation, add 100 µL of reconstituted chromogenic substrate solution to each vial. Cap the vials and swirl gently to mix well. Do not shake or vortex to avoid foaming. Incubate at 37°C±1°C for 6 minutes. 5) Add 500 µL of reconstituted Color-stabilizer #1 (Stop Solution) to each vial and swirl gently to mix well. Do not shake or vortex to avoid foaming. Add 500 µL of reconstituted 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. Bubbles must be avoided. 6) Read the absorbance of each reaction vial at 545 nm using distilled water as blank to adjust the photometer to zero absorbance.
Calculation of Results:	Under the standard conditions, the absorbance at 545 nm shows a linear relationship with the concentration in the range of 0.01 to 1 EU/mL. Plot the absorbance for the four standards on the x-axis and the corresponding endotoxin concentration in EU/mL on the y-axis. Draw a best-fit straight line between these points and determine endotoxin concentrations of samples graphically.
Assay Precision:	Linearity The linearity of the standard curve within the concentration range used to predict endotoxin values need to be verified for each new lot of reagents. At least 4 endotoxin standards spanning the expected concentration range should be assayed along with a blank, in duplicate. 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%.
Restrictions:	For Research Use only

Handling

Storage:	RT/4 °C
Storage Comment:	The kit can be stored dry at room temperature for up to 1 month. For long-term use, the kit can be kept at 2- 8 °C for up to one year. Do not freeze the kit or any of its components.
Expiry Date:	12 months

Publications

Product cited in:

Letsiou, Sammani, Zhang, Zhou, Quijada, Moreno-Vinasco, Dudek, Garcia: "Pathologic mechanical stress and endotoxin exposure increases lung endothelial microparticle shedding." in: **American journal of respiratory cell and molecular biology**, Vol. 52, Issue 2, pp. 193-204, (2015) (PubMed).

Bhatia, Prabhu, Benefiel, Miller, Chow, Davis, Gaskins: "Galacto-oligosaccharides may directly enhance intestinal barrier function through the modulation of goblet cells." in: **Molecular nutrition & food research**, Vol. 59, Issue 3, pp. 566-73, (2015) (PubMed).

Kim, Kim, Kim, Jung, Choi, Kim, Kim: "Myricetin inhibits advanced glycation end product (AGE)-induced migration of retinal pericytes through phosphorylation of ERK1/2, FAK-1, and paxillin in vitro and in vivo." in: **Biochemical pharmacology**, Vol. 93, Issue 4, pp. 496-505, (2015) (PubMed).

Aslani, Schuppe, Guazzone, Bhushan, Wahle, Lochnit, Lustig, Meinhardt, Fijak: "Targeting high mobility group box protein 1 ameliorates testicular inflammation in experimental autoimmune orchitis." in: **Human reproduction (Oxford, England)**, Vol. 30, Issue 2, pp. 417-31, (2015) (PubMed).

Phongsisay, Hara, Fujimoto: "Toll-like receptors recognize distinct proteinase-resistant glycoconjugates in Campylobacter jejuni and Escherichia coli." in: **Molecular immunology**, Vol. 64, Issue 1, pp. 195-203, (2015) (PubMed).

There are more publications referencing this product on: Product page

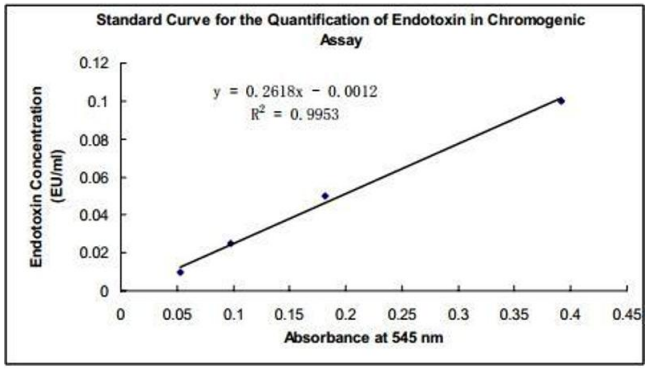

Image 1. If the mean absorbance of a sample is x, the endotoxin concentration of the sample will be (0.2618x – 0.0012) EU/mL. All incubations were performed for 45 min. The figure above shows an example standard curve, the absorption values of standards may be different in different assays.

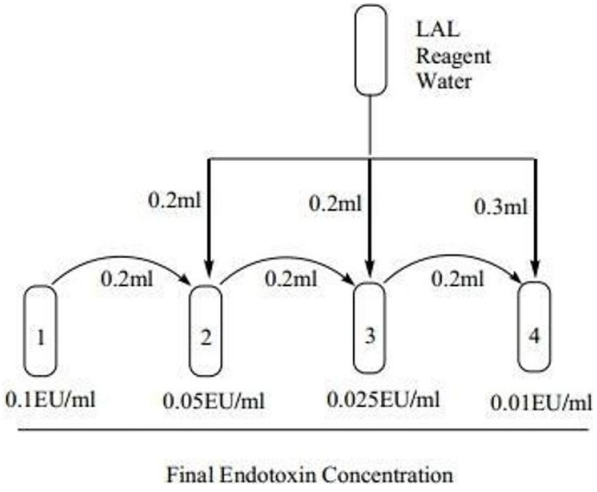

Image 2.