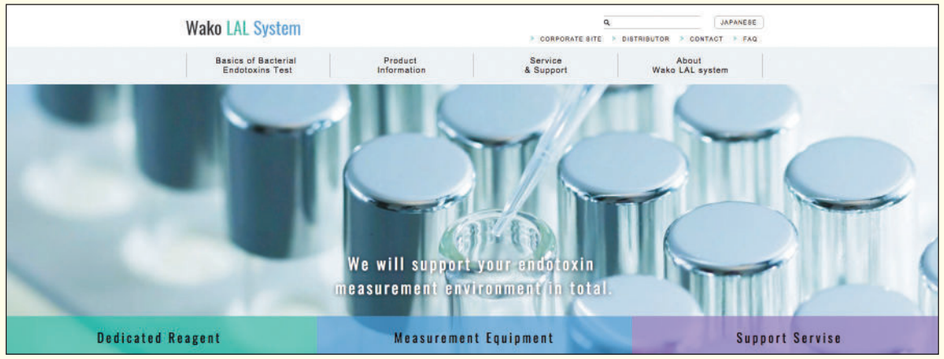
Wako LAL System
Web Site for Endotoxin Analysis



http://www.wako-chem.co.jp/lal/en/index.html

Contact
http://www.wako-chem.co.jp/english/contact.htm

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FUJIFILM
Value from Innovation

Wako

Reagents and Related Products for Detection of Microbial Cell Wall Components

Endotoxin

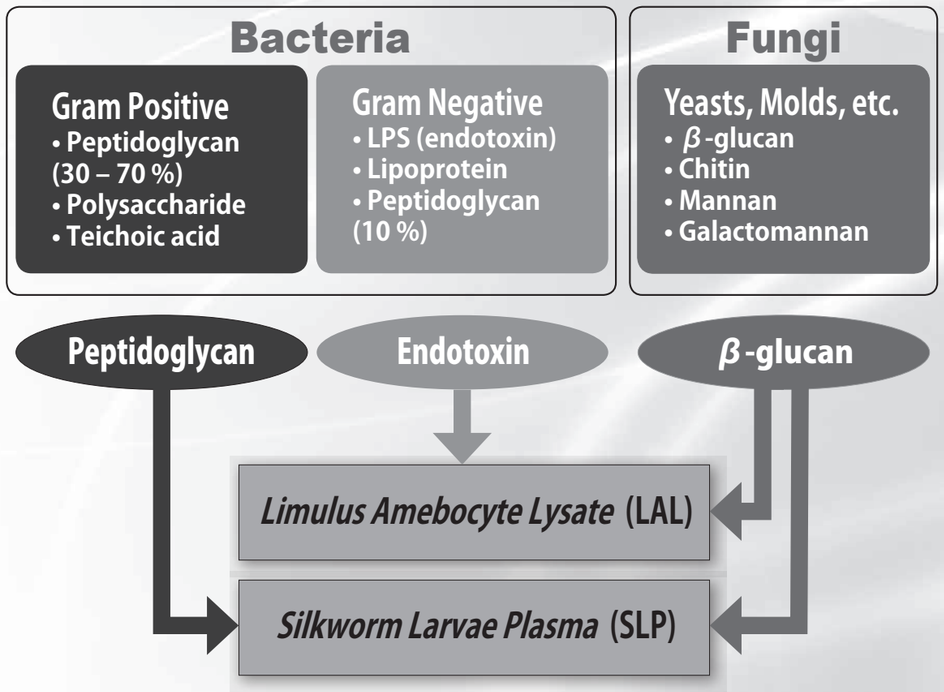
Peptidoglycan

(1 → 3)-β-D-glucan

Revised in April 2018

Contents

Microbial Cell Wall Components



LAL Reagents

●Endotoxin Specific Reagents

Method	Purpose	Type*	Style	Product	Code No.
Turbidimetric technique (Toxinometer®) Gel-clot technique	FDA	Single-test	Kit (with CSE)	Limulus Amebocyte Lysate PYROSTAR™ ES-F SINGLE TEST Labeled sensitivity: 0.015 EU/mL	295-72301
		Multi-test	LAL (without CSE)	Limulus Amebocyte Lysate PYROSTAR™ ES-F MULTI TEST Labeled sensitivity: 0.015 EU/mL	291-75701
	BET (JP)	Single-test	Kit (with CSE)	Limulus ES-Ⅱ Single Test Wako Labeled sensitivity: 0.015 EU/mL	295-51301
		Multi-test	Kit (with CSE)	Limulus ES-Ⅱ Test Wako Labeled sensitivity: 0.015 EU/mL	299-51201
			LAL (without CSE)	Limulus Amebocyte Lysate ES-Ⅱ Labeled sensitivity: 0.015 EU/mL	292-51213
Turbidimetric technique (plate reader)	FDA	Mult-test	Kit (with CSE)	Limulus Amebocyte Lysate PYROSTAR™ ES-F/Plate	293-75401
			LAL (without CSE)	Limulus Amebocyte Lysate PYROSTAR™ ES-F/Plate	297-75301
Chromogenic technique	BET (JP)	Single-test	Kit (with CSE)	Limulus Color KY Single Test Wako	291-53601
		Multi-test	Kit (with CSE)	Limulus Color KY Test Wako	291-53101
Ultra-high-sensitive method with Pyrosep			Without CSE	Limulus PS Single Test Wako	299-54501

SLP Reagent

Peptidoglycan/ β-glucan detection	Toxinometer® specific	Single test	Kit (with PG standard)	SLP-HS Single Reagent Set Sensitivity: 10 pg/mL (PG), 1 pg/mL (β-glucan)	293-58301
	Toxinometer®/ plate reader/ visual test	Multi test	Kit (without standard)	SLP Reagent Set Sensitivity: depends on lot	297-51501

*Single-test: Lysate reagent for single measurment.
The small amount of Lysate reagent is dispensed in each test tube.
Multi-test: Lysate reagent for multiple measurments

Basics of Bacterial Endotoxins Test

1. What is an endotoxin?

An endotoxin is a lipopolysaccharide (LPS) found in the cell wall of gram-negative bacteria. It is a typical pyrogen, which induces various biological reactions when even a small amount of pg (10^{-12} g) or ng (10^{-9} g) enters the bloodstream. Due to its heat resistance and stability, complete inactivation of endotoxin is not possible with autoclaving and dry heat sterilization for at least 30 minutes is required, at a temperature of 250 °C or more. It exists in the environment (e.g. water, air) inhabited by gram-negative bacteria, and bacterial endotoxins (LPS) remain even after the bacteria die.
Figure 1 shows the LPS structure schematic, which illustrates lipid A as the component responsible for the bioactivity. The molecular weight of this portion is approx. 2000. The entire molecular weight, including the sugar chain moiety, is usually approx. 5000 to 8000. However, since an LPS consists of a hydrophilic region (sugar chain) and hydrophobic region (lipid A), it associates in an aqueous solution to form a micellar structure with apparent molecular weight of hundreds of thousands to several millions. A change in the micellar structure reportedly influences the strength of bioactivity.
Figure 2 illustrates the structures of *Salmonella*-type and *E. coli*-type lipid A, which indicate that the basic structure of the lipid A is maintained fairly well, regardless of the strain variation.

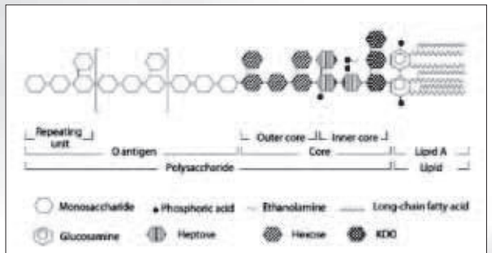


Figure 1: Lipopolysaccharide Structure Schematic Diagram

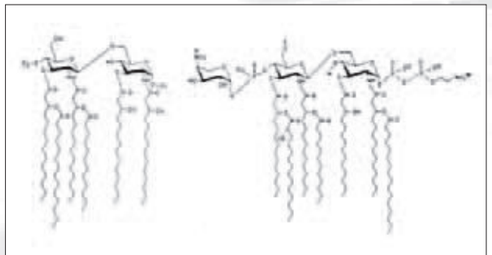


Figure 2: Lipid A Structure (*E. coli* and *Salmonella* types)

2. Various Endotoxin Test Methods Using LAL Reagents

A lysate reagent prepared from the amebocytes of horseshoe crab (*Limulus polyphemus*) is used to detect bacterial endotoxins. As shown in Figure 3, the cascade reactions start by the presence of an endotoxin, whereby Factor C, a serine protease precursor, is initially activated. There follows the subsequential activation of Factor B, also a serine protease precursor and a proclotting enzyme, which hydrolyzes coagulogen into coagulin, forming an insoluble gel. In LAL tests, endotoxin can be quantified in three ways: measurement of gel formation, increased turbidity, or a yellow chromogen released due to the synthetic substrate cleavage.

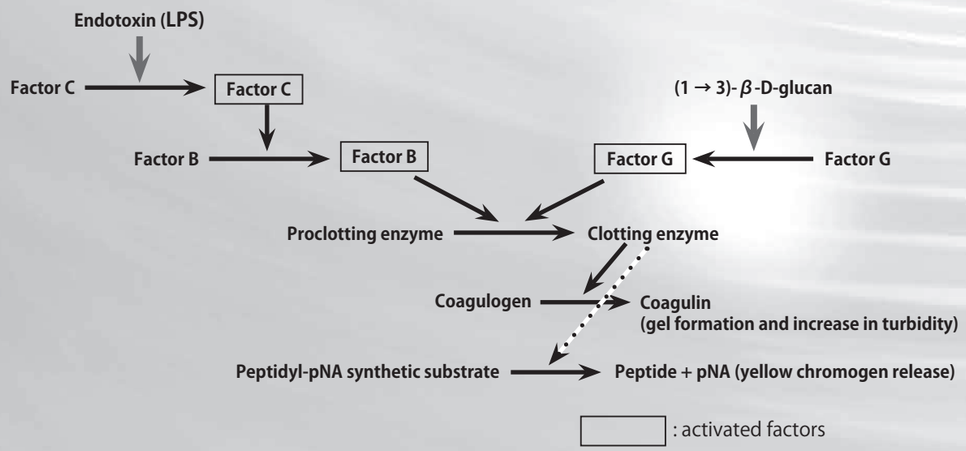
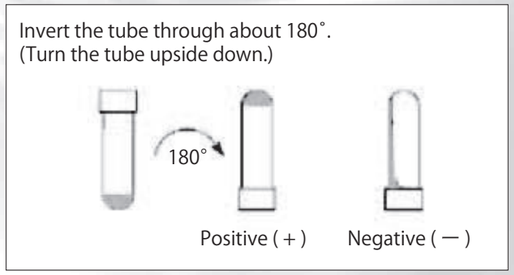


Figure 3: LAL Reagent Reaction Mechanism

Ordinary LAL reagents react not only with the endotoxin but also (1→3)-β-D-glucan (a fungal cell wall component), since the Factor G pathway can be activated in the reagents. To eliminate this (1→3)-β-D-glucan activation, various endotoxin-specific reagents are being developed in industry by removing Factor G or inhibiting its activation.
Various LAL reagents are commercially available, as well as measuring systems based on the Figure 2 reaction mechanism. It is essential to select the most appropriate product depending on the required accuracy, test frequencies, number of samples and other relevant factors.
Pharmacopoeias in the U.S., Europe and Japan refer to three means of endotoxin detection, namely the gel-clot technique, chromogenic and turbidimetric techniques, which are detailed in the following sections while introducing the characteristics and application examples of our relevant LAL reagents.

(1) Gel-clot Technique

Mix a sample with LAL reagent in a test tube and incubate it using a block heater at 37 ± 1 °C, 60 ± 2 minutes, without subjecting to vibration. Upon completion of heating, immediately but slowly tilt the tube through 180°. If a gel has formed and maintains its integrity without deformation or collapse, the result can be determined positive, while it is negative if no gel has formed. During the test, a series of samples is diluted multiple times (usually 2-fold) to check if the result is positive in each sample. The maximum valid dilution or the minimum concentration determined positive is referred to as the endpoint.



Corresponding Reagent:

The reagent is available in a single-type kit with reaction vials containing pre-dispensed reagent for a single measurement, and a multi-type kit for dispensing the required amount of the dissolved reagent into reaction vials. The single-type kit is ideal for an assay with a few samples, and the multi-type kit for a larger number of samples.
A multi-type kit is used by dispensing 0.1 mL of dissolved LAL reagent into reaction tubes, which is then mixed after having 0.1 mL of the sample added. A single-type kit can be used by adding 0.2 mL of the sample to the reaction vial with pre-dispensed, lyophilized LAL reagent.

ES-II Series

Endotoxin-specific LAL reagents (not activated by (1→3)-β-D-glucan), compatible with the BET (JP) compliance tests. They have the gelation sensitivity of 0.015 EU/mL, and are available in both single- and multi-type kits.

*BET: Bacterial Endotoxins Test

(2) Chromogenic Technique

This technique uses synthetic chromogenic substrate cleavage to detect the activation of LAL reagent induced by endotoxin. Since the yellow color of p-nitroaniline is measured by absorbance at approx. 405 nm, the technique is not applicable if the sample has considerable absorbance at approx. 405 nm.

Corresponding Reagent:

Color KY Series

Endotoxin-specific chromogenic technique LAL reagents, compatible with the BET* (JP) compliance testing. A single-type kit combined with a Toxinometer® and a multi-type kit for use in combination with a microplate reader and Toxinometer® are available for kinetic chromogenic testing. These series also feature measurements at the lowest concentration (highest sensitivity) among our reagent products: detection limit of 0.0002 EU/mL (single-type) and 0.0005 EU/mL (multi-type).

(3)Turbidimetric Technique

This technique uses the change in gel turbidity to detect the activation of LAL reagent induced by endotoxin. It cannot be applied to samples with considerable turbidity.

Corresponding Reagent:

ES-F Series and ES-II Series

Kinetic turbidimetric measurement is available by combining the ES-F series or the ES-II series and Toxinometer® or a microplate reader.These reagent kits can provide gel-clot results while obtaining kinetic turbidimetric data, with the measurement time specified as 60 minutes.

3. Tools Used for Testing

All tools used for endotoxin detection must be free from endotoxin and β-glucan. Dry-heating at 250 °C for more than 30 minutes is required to deactivate endotoxin. The use of glassware treated by dry heat sterilization is recommended. Avoid metal tools, since even a small amount of eluted metal ions (e.g. Fe, Al, Ga, Cr) may affect testing. When using disposable plastic tools (not guaranteed by its manufacturer for use for testing purposes), check if they satisfy the requirements: 1) Not contaminated by endotoxin; 2) No adsorption of endotoxin; and 3) No eluted substance; in comparison with glassware.

4. Reference Standard Endotoxin

Use the appropriate type of standard endotoxin based on the testing purpose.

- Tests in compliance with BET (USP/EP/JP) such as final product inspection of pharmaceuticals and medical devices
  → Reference Standard Endotoxin of USP, EP or JP must be used.
- Tests for inspecting materials, processes and other related subjects
  → Control Standard Endotoxin (CSE) can be used.

*BET: Bacterial Endotoxins Test

5. Interference of Sample

Precautions are required against the potential impact (reaction interferences) of samples on endotoxin tests. These interferences are categorized into the following two types:

❶ Influence on LAL Reagent

- Protein denaturants (e.g. acids, alkalis, urea, surfactants, organic solvents)
- Protease and protease inhibitors
- Chelating agents (whereby Ca and Mg required for reactions are scavenged)
- For the chromogenic technique: coloring matter (substance with considerable absorbance at approx. 405 nm)
- For the turbidimetric technique: turbidity

❷ Influence on Endotoxin

- Metal ions (e.g. Fe, Al, Ga and Cr ions. Influential even at a micromole level)
- Surfactants

The effect of samples can be judged by a test referred to as the test for interference factors by Pharmacopoeias: namely, it is conducted by measuring a sample which has been spiked with a known amount of endotoxin and obtaining the recovery of the spiked endotoxin. If the recovery is within the 50 to 200 % range, then the sample is determined as not influential, in other words, the measured endotoxin concentration is correct. If any sample influence is found, it can be reduced by diluting the sample solution for measurement. However, dilution of the sample solution raises the endotoxin concentration value obtained through the conversion to the concentration of the original solution (pre-dilution solution). The possible dilution multiple (maximum valid dilution) is determined based on the desired endotoxin concentration to be detected and the detection sensitivity of the LAL reagent to be used (refer to the Bacterial Endotoxins Test by the Pharmacopoeia for details of the reaction interference factors and maximum valid dilution).

6. Principle of Limulus ES (Endotoxin Specific Reagent)

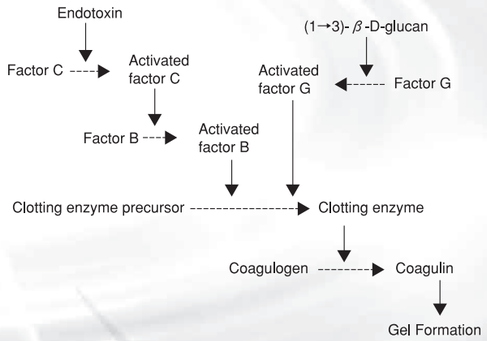
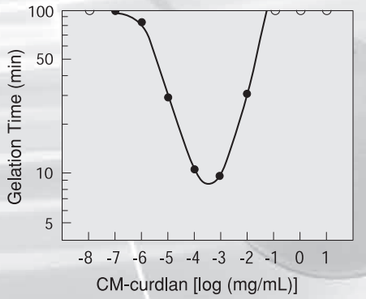


Figure 1: Limulus Test Reaction Cascade

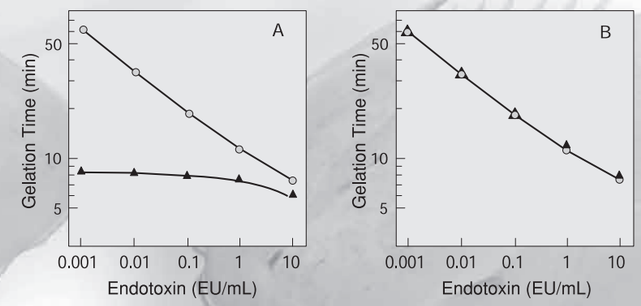
The reaction cascade mechanism of the LAL reagent and endotoxin is shown in Figure 1. If (1 → 3)-β-D-glucan* exists in the reaction system, it activates Factor G, causing a false positive reaction in which gelation occurs. This happens regardless of the presence of endotoxin, meaning endotoxin specific detection is not available. Wako's Limulus ES has been developed to inhibit the interference of (1 → 3)-β-D-glucan, by making an excessive amount of (1 → 3)-β-D-glucan (carboxymethylated curdlan) coexistent in the reaction system. Thus, the activation of LAL reagent by β-glucan is inhibited, enabling endotoxin specific detection.
The reason why an excessive amount of β-glucan can inhibit its own reaction is shown in Figure 2: the reaction range between the β-glucan and LAL reagent is too narrow for reaction. On the other hand, the reaction of endotoxin and Limulus occurs over a wide range of concentration and is not subject to any interference from the large amount of coexisting β-glucan. Wako's Limulus ES use this principle (see Figure 3).

*(1 → 3)-β-D-glucan derived from mold (fungus) or cellulosic membrane filter.



Measured by using a Toxinometer® (with the measurement time set to 99 minutes) and Limulus HS
○ : Gelation was not determined within 99 minutes
● : Gelation was determined

Figure 2: CM-curdlan and LAL Reaction



A: Limulus HS
B: Limulus ES
○ : Endotoxin dilution series
▲ : Endotoxin dilution series containing 1 μg/mL CM-curdlan

Figure 3: Effect of CM-curdlan on Endotoxin Measurement Using Limulus HS and Limulus ES

PYROSTAR™ ES-F series

Turbidimetric Technique | Gel-clot Technique | Endotoxin Specific | FDA approved

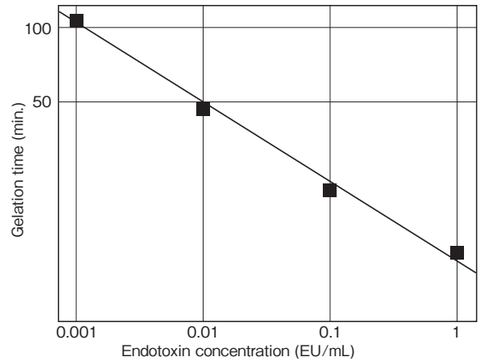
■ Features

- It is possible to specifically detect endotoxin without it being affected by (1 → 3) - β-D - Glucan in the sample.
- US Food and Drug Administration (FDA) approved .
- Applicable kits and reagents in the final product testing of US exports.
- Gelation sensitivity test value (EU / mL) by USP Reference Standard Endotoxin (USP - RSE) is indicated on label.
- Turbidimetric Technique with Toxinometer ® can detect up to 0.001 - 1 EU / mL.
- Stable endotoxin measurement is possible, due to the minor pH impact of the measurement sample on testing.

Single-test			
Code No	Product Name	Labeled Gelation Sensitivity (EU/mL)	Quantity
with CSE 295-72301	Limulus Amebocyte Lysate PYROSTAR™ ES-F SINGLE TEST	0.015	25 tests

Multi-test			
291-75701	Limulus Amebocyte Lysate PYROSTAR™ ES-F MULTI TEST without CSE	0.015	100 tests (2.0mL×5vials)

■ standard curve information



Regression equation: log(Tg) = -0.289200 log(C) +1.13012
Correlation coefficient: -0.9984

Endotoxin concentration (EU/mL)	Gelation time (min.)
0.001000	103.8
	103.8
	103.6
0.01000	49.2
	49.0
	48.8
0.1000	25.2
	25.2
	25.2
1.000	14.0
	14.0
	14.2

■ Recovery rate of spiked endotoxins with the Turbidimetric technique

Single test

Coexisting drug	0	50	100	150(%)
0.9% Sodium Chloride	74			
3% Sodium Chloride	39			
2.5% Glucose	80			
Ringer Solution (undiluted)	87			
Magnesium Sulfate (50mM)	72			
Iothalamate Meglumine (1.0%)	69			
Sodium Bicarbonate (0.25%)	89			
Sodium Citrate (0.05%)	108			
Sodium Heparin (10U/mL)	97			
Xylitol (2.5%)	82			
Endotoxin Extract (0.1%)	77			
Pyridoxine Hydrochloride (0.25mg/mL)	104			
Sodium Ascorbate (10mg/mL)	102			
Cefozopran Hydrochloride (2.5mg/mL)	83			

Multi test

Coexisting drug	0	50	100	150(%)
0.9% Sodium Chloride	105			
3% Sodium Chloride	88			
2.5% Glucose	98			
Ringer Solution (undiluted)	103			
Magnesium Sulfate (50mM)	100			
Iothalamate Meglumine (1.0%)	89			
Sodium Bicarbonate (0.25%)	126			
Sodium Citrate (0.1%)	93			
Sodium Heparin (10U/mL)	92			
Xylitol (2.5%)	91			
Endotoxin Extract (0.1%)	90			
Pyridoxine Hydrochloride (0.25mg/mL)	100			
Sodium Ascorbate (25mg/mL)	74			
Cefozopran Hydrochloride (5mg/mL)	85			

* Multi test is performed by mixing 100 μL of lysate reagent with 100 μL of sample volume, whereas in single test, a 200 μL sample directly dissolves lysate reagent. Therefore, there will be a difference in sample concentration in the reaction solution. In general, single tests tend to be more affected by samples.

PYROSTAR™ ES-F/Plate

Turbidimetric Technique | Endotoxin Specific | FDA approved

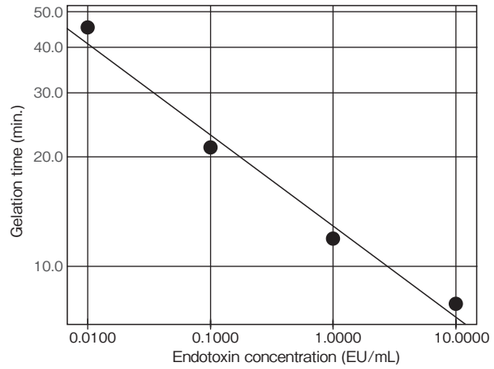
PYROSTAR ™ ES-F / Plate is a specific Turbidimetric lysate reagent specifically for 96-well microplates.
By using an absorbance microplate reader, simultaneous measurement of multiple samples is easily made possible.
The quantitative range is wide, from 0.01 to 10 EU / mL, and it can be applied to water type tests, as well as to the testing of name drugs that require dilution.

■ Features

- It is possible to specifically detect endotoxin without it being affected by (1 → 3) - β-D - Glucan in the sample.
- US Food and Drug Administration (FDA) approved .
- Regarding U.S. exports, it can also be applied to the final product testing.
- Since it can be measured with a sample volume of 50 μL per well, even a precious sample can be measured in a small amount.
- Stable endotoxin measurement is possible, due to the minor pH impact of the measurement sample on testing.

Multi-test			
Code No	Product name	Quantitative Range (EU/mL)	Quantity
with CSE 293-75401	Limulus Amebocyte Lysate PYROSTAR™ ES-F/Plate with CSE	0.01-10	160 tests (2.0mL×4vials)
297-75301	Limulus Amebocyte Lysate PYROSTAR™ ES-F/Plate without CSE	0.01-10	200 tests (2.0mL ×5vials)

■ standard curve information



Regression equation: log(Ta) = -0.260424 log(C) +1.10351
Correlation coefficient: -0.9943

Endotoxin concentration (EU/mL)	Gelation time (min.)
0.0100	46.0
	46.7
	43.3
0.1000	21.2
	21.5
	21.2
1. 000	12.0
	12.1
	11.9
10.000	7.4
	7.4
	7.5

■ Recovery rate of spiked endotoxins with the Turbidimetric technique

Coexisting drug	0 50 100 150 (%)
0.9% Sodium Chloride	94
3% Sodium Chloride	81
2.5% Glucose	105
Ringer Solution (undiluted)	106
Magnesium Sulfate (50mM)	96
Iothalamate Meglumine (1.0%)	87
Sodium Bicarbonate (0.25%)	127
Sodium Citrate (0.05%)	90
Sodium Heparin (10U/mL)	96
Xylitol (2.5%)	89
Endotoxin Extract (0.1%)	87
Pyridoxine Hydrochloride (0.25mg/mL)	103
Sodium Ascorbate (10mg/mL)	65
Cefozopran Hydrochloride (2.5mg/mL)	85

Limulus ES-Ⅱ Series

Endotoxin Specific Limulus Reagent

Turbidimetric Technique | Gel-clot Technique | Endotoxin Specific

■ Features

- Highly sensitive endotoxin specific detection without the interference of (1 → 3)-β-D-glucan in the sample is possible.
- Kit and reagent compatible with the Bacterial Endotoxins Test specified in BET (JP)
- The LAL reagent is labeled with gelation sensitivity in titers (EU/mL) measured using Reference Control Endotoxin of JP.
- Firm gel formation capability to facilitate determination of gelation
- A multi-test type requires the dissolution of the reagent in water for the endotoxin test, while a single-test type only requires the addition of 0.2 mL of sample.
- Highly sensitive analysis is available using a Toxinometer®.
- Stable endotoxin measurement is possible, due to the minor pH impact of the measurement sample on testing.
- Turbidimetric Technique with Toxinometer®can detect from 0.0078 to 1 EU/mL.

Single-test			
Code No.	Product Name	Labeled Gelation Sensitivity (EU/mL)	Quantity
with CSE 295-51301	Limulus ES-Ⅱ Single Test Wako	0.015	25 tests

Kit: Limulus ES-Ⅱ reagent, 1 sample for 0.2 mL × 25 vials; Control Standard Endotoxin (CSE) × 1 vial

Multi-test			
with CSE 299-51201	Limulus ES-Ⅱ Test Wako	0.015	60 tests

Kit: Limulus ES-Ⅱ reagent for 2 mL × 3 vials; Control Standard Endotoxin (CSE) × 1 vial
LAL reagent solution can be stored below −20 ℃ for up to 2 weeks.

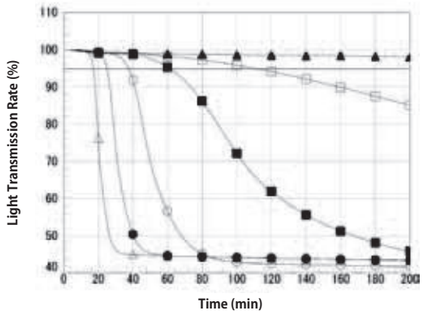
Multi-test			
292-51213	Limulus Amebocyte Lysate ES-Ⅱ, Lyophilized	0.015	100 tests

Kit: ES-Ⅱ reagent for 2 mL × 5 vials

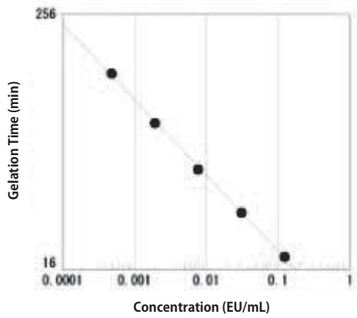
The Control Standard Endotoxin mentioned in the kits above is a lyophilized product, purified from *E. coli* UKT-B, containing 500 ng endotoxin, as well as mannitol and glycine as additives in each vial. Each lot is labeled with a typical value (EU/vial) measured by using Reference Control Endotoxin of JP.

■ Experiment Example

The measurement is performed for 200 minutes and analyzed via a highly sensitive analysis method using a Toxinometer® and Limulus ES-Ⅱ Single Test Wako. The standard curve obtained shows a high correlation within a wide concentration range from 0.0078 to 0.125 EU/mL.



Time Course Data



Standard Curve Data

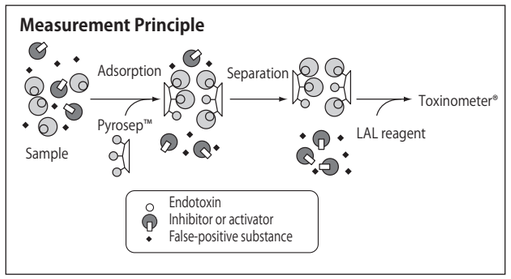
Limulus PS Single Test Wako

■ Features

- Endotoxin specific measurement can be performed with small amounts, avoiding any interference from coexisting substances, by adsorbing endotoxin to Pyrosep™ in the sample and condensing it.
- Since the endotoxin is adsorbed to Pyrosep™ even in ethanol, a fat-soluble sample can be tested provided it dissolves in ethanol. Such samples are not usually processed in ordinary limulus tests. (Example: fat-soluble vitamins, oils and fats)
- No LAL reagent is wasted, since it is pre-dispensed individually in vials, and can be used in the quantity required for measurement.

■ Measurement Principle

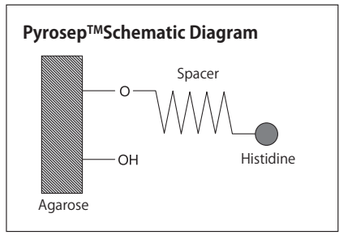
The endotoxin in the sample is adsorbed to a capillary column filled with Pyrosep™(an affinity adsorbent with histidine bound to a water-insoluble support via a spacer, designed to specifically adsorb endotoxin). Other substances contained in the sample are washed off, allowing the adsorbed endotoxin activate the LAL reagent for quantification, whereupon the measurement is performed using a Toxinometer®.

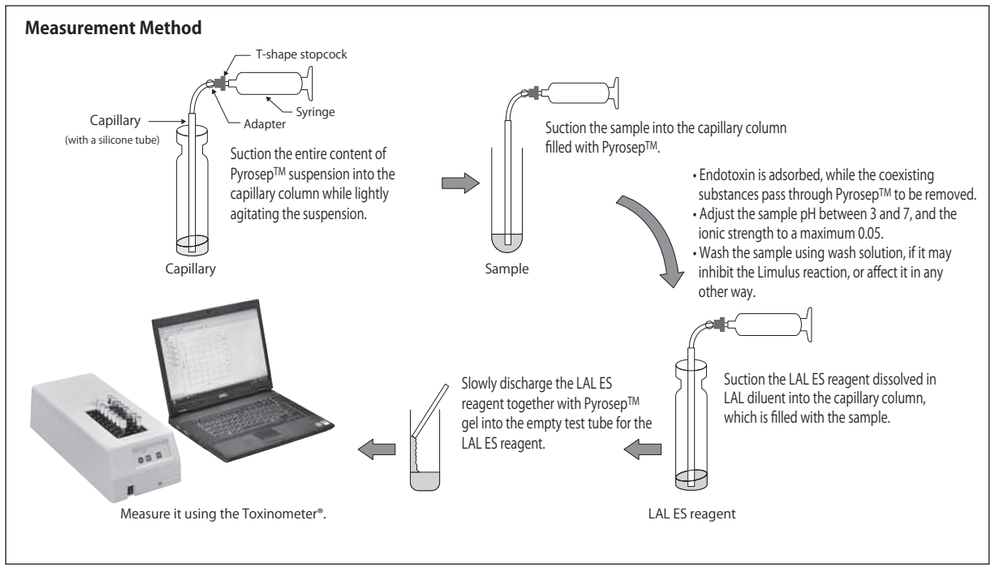


■ Kit Contents

(1) LAL ES Single reagent (lyophilized Limulus polyphemus amebocyte extract)	20 vials (for 0.3 mL)
(2) Pyrosep™ suspension (suspended in a phosphate buffer)	20 vials (0.77 mL)
(3) LAL diluent	2 vials (7 mL)
(4) Wash solution	4 vials (11 mL)
(5) Sample dilution buffer	4 vials (10 mL)
(6) Capillary (with a silicone tube)	20 pcs

• The syringe, T-shaped stopcock and dedicated adapter are not included in this kit, but are optionally available in the PS Accessory Kit (294-33311).





■ Application

A. Endotoxin Detection in Dialysate

The samples were prepared from commercially-available dialysate (dialysate X) and two kinds of replacement fluids for artificial kidneys (fluids Y and Z), with amounts of 5.0 mL in each case respectively. Each had USP Reference Standard Endotoxin added, and the pH was subsequently adjusted to approx. 5 using acetic acid. The endotoxin concentration in each sample was measured using the Pyrosep™ method and the added endotoxin was recovered.
With the use of the Pyrosep™ method, endotoxin in dialysate is detectable within a range starting from 0.1 EU/L.

Standard curve (sample volume: 5.0 mL)

No.	Specified Concentration (EU/L)	Tg(min)	Average Tg (min)
1	1.000	53.0	53.60
		53.4	
		54.4	
2	2.000	41.6	41.53
		41.2	
		41.8	
3	10.00	26.6	26.07
		26.0	
		25.6	
4	100.0	14.6	14.60
		14.8	
		14.4	

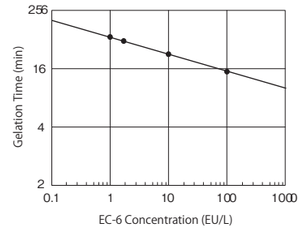


Table 1: Endotoxin Recovery from Peritoneal Dialysate/Replacement Fluid Using the Pyrosep ™ Method

Sample	Endotoxin Spike (EU/L)	Endotoxin Recovery (%)
Dialysate X	0.1	80
	1.0	72
Fluid Y	0.1	144
	1.0	102
Fluid Z	0.1	108
	1.0	118

B. Endotoxin Detection in Fat-soluble Vitamin

Fat-soluble vitamins D, E and K were dissolved in ethanol, to which USP Reference Standard Endotoxin was then added. The endotoxin concentrations in the samples were measured using the Pyrosep™ method and the added endotoxin was recovered from each sample. The use of the Pyrosep™ method enabled the endotoxin detection in fat-soluble vitamins, which could not otherwise be measured with Limulus reagents.

Table 2: Endotoxin Recovery from Fat-soluble Vitamins Dissolved in Ethanol Using the Pyrosep™ Method

Vitamin	Concentration (mg/mL)	Endotoxin Recovery (%)
D_2	10	170
E	10	91
	20	83
K	10	99
	20	68

• Endotoxin addition amount/concentration adjusted to make the final concentration 0.05 EU/mL.

C. Other Examples

Ethanol, silicone oil, soybean oil, olive oil, stearic acid, egg yolk lecithin, oil-adjuvanted vaccine, albumin products, coagulation factor products (factor 8), interferon formulation, antithrombin Ⅲ, immunoglobulin product and other ethanol-soluble samples

Single-test		
Code No.	Product Name	Quantity
299-54501	Limulus PS Single Test Wako	20 tests

Accessory Kit		
294-33311	PS Accessory Kit	1 set

Kit contents:
20 mL syringe (no needle) × 12, T-shape stopcock R type × 12, dedicated adapter × 20, bulldog clip × 12, 352058 FALCON polystyrene tube (5 mL) × 25, 352057 FALCON polystyrene tube (14 mL) × 25, test tube rack (for 50 tubes) × 1 and procedure instruction document × 1

Chromogenic Technique (Kinetic Yellow Synthetic Substrate Method)

Limulus Color KY Series

Endotoxin Specific Limulus Reagent

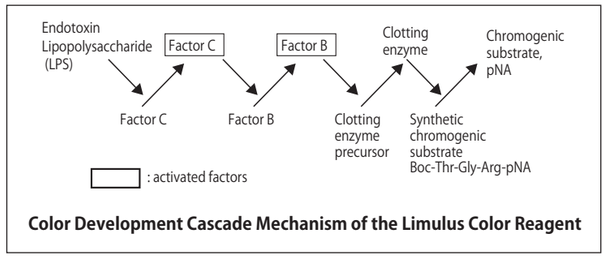
Chromogenic Technique | Endotoxin Specific

■ Features

- Prepared with LAL reagent and a synthetic chromogenic substrate pre-dispensed in individual units, for a simple procedure by mixing the dissolved reagent with the sample (no reagent dissolution is required with single-test type)
- Excellent stability of the dissolved reagent suppresses the reaction of the blank solution.
- Endotoxin specific detection without the interference of (1 → 3)-β-D-glucan is possible.
- A wide determination range eliminates sample dilution in many tests.
- Thanks to the high sensitivity performance, the effect of reaction accelerators or inhibitors can be reduced by diluting the samples.
- Quantification using a Toxinometer® or a microplate reader is possible with multi-test type.

■ Principle

The reagent is based on the color development mechanism, which is activated by the presence of endotoxin. First, a series of activations of serine protease precursors in the reagent occur, consequently triggering the final reaction, in which a clotting enzyme hydrolyzes the chromogenic substrate to release a yellow chromogen (pNA).



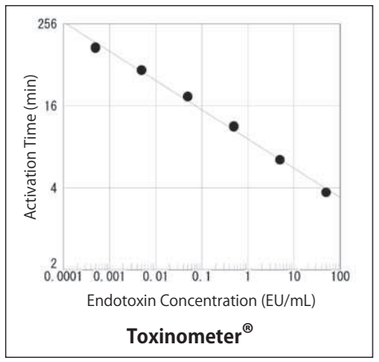
Color Development Cascade Mechanism of the Limulus Color Reagent

Single-test (Toxinometer® Assay)			
Code No.	Product Name	Quantitative Range (EU/mL)	Quantity
291-53601	Limulus Color KY Single Test Wako	0.0002-5	25 tests

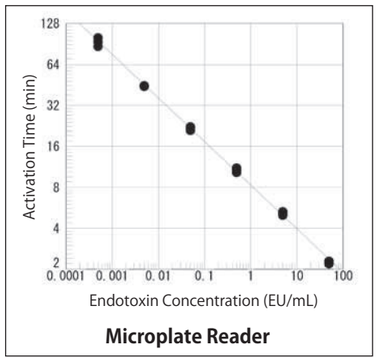
Kit: Limulus Color reagent, 1 sample for 0.2 mL × 25 vials; Control Standard Endotoxin (CSE) × 1 vial

Multi-test			
291-53101	Limulus Color KY Test Wako	0.0005-5	60 tests

Kit: Limulus Color reagent for 2 mL (20 tests with Toxinometer®/40 tests with microplate reader) × 3 vials; Control Standard Endotoxin (CSE) × 1 vial
LAL reagent solution can be stored below −80 ℃ for up to 2 weeks.



Toxinometer®

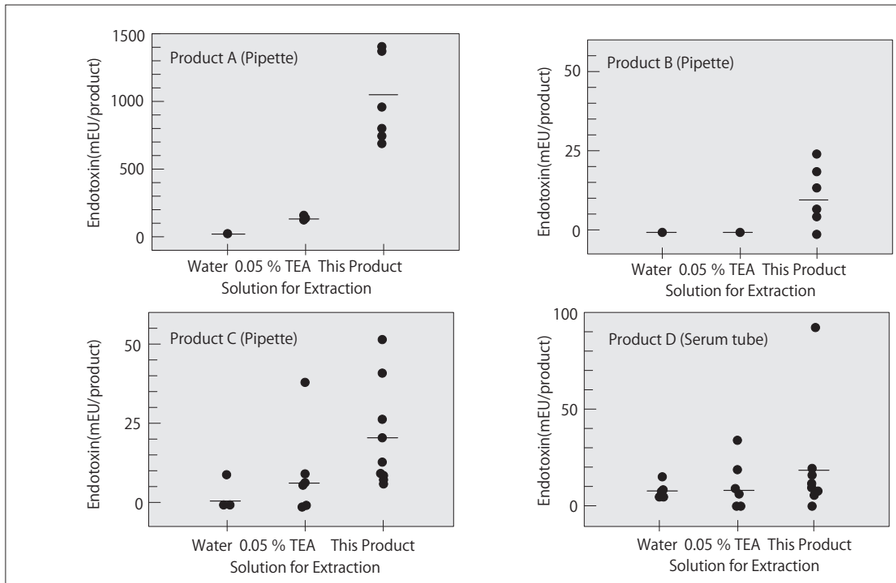


Microplate Reader

Ideal for Endotoxin Tests on Medical Devices and Equipment

Endotoxin Extracting Solution for LAL Test

Conventionally, water or saline solution has been used to extract endotoxin in tests on medical devices and equipment. However, the efficacy of this extraction method is now in question. This product is an endotoxin-free extracting solution (to be used in 1:20 dilution) containing human serum albumin (HSA), and capable of extracting endotoxins which cannot be extracted in water or saline solution. It is recommended that this product be used in particular for endotoxin testing on equipment and devices which may come into contact with fluids containing blood or protein.



Endotoxin Contamination Detection Using Various Extracting Solutions on Commercially Available Sterilized Equipment/Devices

■ Usage

1. Prepare Endotoxin Extracting Solution
   Dilute a single vial of the product (10 mL) into 190 mL of endotoxin-free water to a 1:20 concentration.
2. How to Use
   Use a certain amount of the prepared endotoxin extracting solution and either add it to the sample, or dip the sample in it. Then, lightly agitate the solution and incubate it at room temperature for approx. one hour, whereupon it should be gently re-agitated and the extracted endotoxin measured.

Code No.	Product Name	Application	Quantity
293-51601	Endotoxin Extracting Solution for LAL Test	For endotoxin detection (for 1:20 dilution)	10 mL × 4

■ References

1) Katsuhito Inoue, Aya Takaoka, Masakazu Tsuchiya, Shuji Matsuura: Excerpt 110 (1992), the 6th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments
2) Katsuhito Inoue, Tomoaki Harada, Masakazu Tsuchiya, Shuji Matsuura: Excerpt 44 (1994), the 21st Annual Meeting of the Society for Antibacterial and Antifungal Agents

SLP Reagent Set

Silkworm Larvae Plasma Reagent

Research Use only,Not for use in diagnostic procedures

Principle

The hemolymph of the silkworm (*Bombyx mori*) contains a self-defense mechanism termed "the prophenoloxidase cascade system (ProPO)," which is triggered by peptidoglycan (PG) and (1 → 3)-β-D-glucan (β-glucan), consequently activating prophenoloxidase (PO) in the system. It is postulated that serial serine proteases are involved in the PO activation; however, this has not yet been elucidated. This cascade system is believed to participate in the melanin formation observed in the insect's body fluids as a self-defense mechanism.

SLP reagent is a lyophilized product prepared under sterile conditions from the silkworm hemolymph, which contains all the ProPO factors involved in the cascade system. The reagent, activated by PG and β-glucan, oxidizes the DOPA (L-3,4-dihydroxyphenylalanine) in the substrate and forms a melanin pigment. Since PG is a component found in most bacterial cell walls and β-glucan, in many fungal cell walls, SLP enables the detection of various microorganisms by measuring the melanin pigment formation.

The activation mechanism of SLP is shown in Figure 1. PG or β-glucan binds to the respective recognition protein (PGRP or GRP), which initiates the ProPO cascade reactions, and consequently activates prophenoloxidase. The activated prophenoloxidase then oxidizes the DOPA in the substrate, thus forming black melanin pigment. Since endotoxin, which can be detected by horseshoe crab (*Limulus polyphemus*) amebocytes (LAL reagent), does not activate the SLP cascade system, it cannot be detected with SLP. However, it can detect PG, which cannot be detected by the LAL reagent (Figure 1).

The SLP reagent strongly reacts with PG and β-glucan with β-1,3-glucoside bond, derived from various bacteria. However, it is barely activated by lipopolysaccharide (LPS) (endotoxin), a cell wall component of gram-negative bacteria.

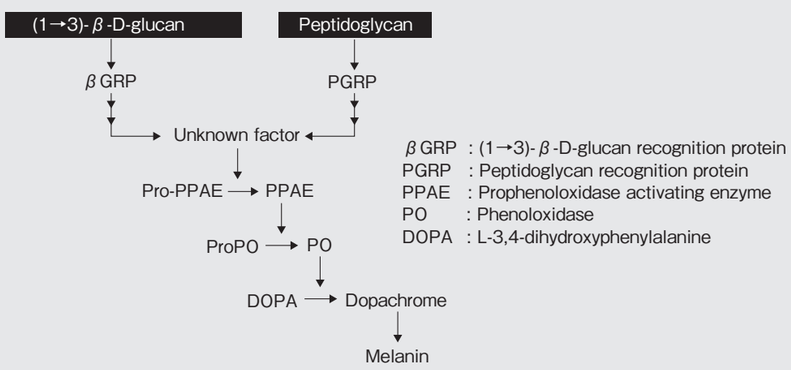


Figure 1: Silkworm Hemolymph Prophenoloxidase Cascade System

Since PG is found not only in gram-positive but also gram-negative bacteria, the SLP reagent responds to a wide range of bacteria, regardless of their gram-staining classification. It also reacts with fungus-derived β-glucan, which suggests that it can respond widely to general microorganisms. Therefore, the concurrent use of LAL and SLP reagents, which are activated by endotoxin and β-glucan respectively, enable the type of microorganism in a sample to be predicted.

■ Features

1. Sensitive quantitation of peptidoglycan and β-glucan
2. Visual detection without the use of any special apparatus is possible.
3. Sensitive and accurate quantification of peptidoglycan and β-glucan is possible by using a microplate reader or Toxinometer®, a parallel turbidimetric time analyzer.
4. Stable for a minimum 18 months, in lyophilized (2 to 10 ℃) form

■ Applications

1. Elucidation of the peptidoglycan structure-activity relationship, biosynthesis, metabolism and pathogenic significance
2. Water contamination measurement
3. Microbial contamination testing of the dialysis solution
4. Detection for fungal compounds in pharmaceuticals and medical devices, biologics and genetically-engineered products
5. Elucidation of the biological defense mechanism of insects

■ Kit Contents

● SLP Reagent Set

1. SLP reagent 3 mL × 1 vial
   Sensitivity: Endpoints (PG and β-glucan) determined by a visual test (after 60-minute incubation at 30 ℃) indicated for each lot
2. Substrate 3 mL × 1 vial
   DOPA contained as a substrate
3. Substrate diluent 4 mL × 1 vial
   Good's buffer contained

● SLP-HS Single Reagent Set

1. SLP-HS (Silkworm Larvae Plasma High Sensitive) 0.2 mL × 20 vials
   Sensitivity: 10 pg/mL (PG), 1 pg/mL (β-glucan) (detected within 120 minutes with Toxinometer® at 30℃)
2. SLP diluent 1.0 mL × 20 vials
3. Standard (digested peptidoglycan from S. aureus) 0.5 mL × 1 vial

Products			
Code No.	Product Name	Application	Quantity
297-51501	SLP Reagent Set	For microorganism detection	3 mL
293-58301	SLP-HS Single Reagent Set	For microorganism detection	20 tests

Measurement Method

Several methods for measuring PG and β-glucan using SLP are available: Toxinometer® measurement, microplate reader measurement and visual determination.
The method using a microplate reader or Toxinometer® uses the pigment quantity (absorbance change) generated by the activation, and measures the activation time (Ta or onset time) of the reactions from the start to the point at which the absorbance reaches its predetermined threshold, quantifying the SLP activation substance (PG or β-glucan). The principles used in these methods are the same as those for measuring endotoxin using a Toxinometer® (see Figure 2). Figure 4 shows a measurement example using a Toxinometer®, while Figure 3 shows the microplate reader measurement principle and Figure 5, an example of the same.
The visual determination refers to the procedure of checking the color change observed in the reaction mixture after a certain reaction period has elapsed. This visual determination method is similar to that used in the LAL gelation test; however, this is easier and more reliable, since the color change is more distinct and there is no vibration influence.

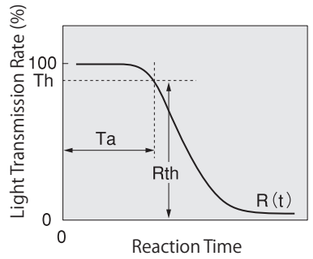


Figure 2: Principle: Measures Ta from the Measurement Start to the Point at Which R(t) Reaches Rth (Th: Threshold)

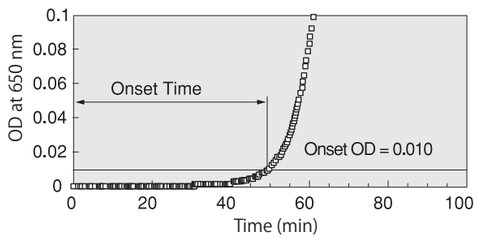


Figure 3: Principle: Measures the Time Elapsed from the Measurement Start to the Point at Which OD is 0.01

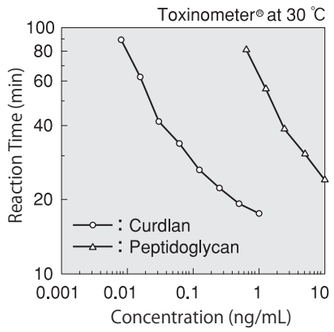


Figure 4: Method Using a Toxinometer®

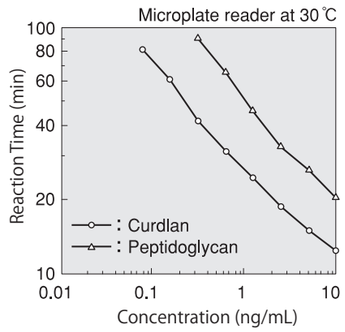


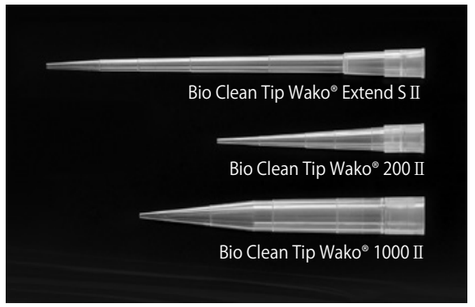
Figure 5: Method Using a Microplate Reader

Bio Clean Series

■ Features

- Endotoxin-free
- Individually packaged
- Ideal for use in clean rooms, thanks to the packaging film featuring dust-free protection
- Sterilized by gamma irradiation

Bio Clean Tip Wako®



Bio Clean Plate Wako™

Code No.	Product Name	Size	Quantity
294-35011	Bio Clean Tip Wako® Extend S II	200 μL	100 pcs
291-35021	Bio Clean Tip Wako® 200 II	200 μL	100 pcs
298-35031	Bio Clean Tip Wako® 1000 II	1000 μL	100 pcs
293-35221	Bio Clean Plate Wako™	96well	50plates

Consumables

Test Tube for Endotoxin Test, Aluminum Cap and Whole Pipette

Code No.	Product Name	Size	Quantity
292-32751	Limulus Test Tube-S with Aluminum Cap	φ12 × 75 mm	10 pcs × 8
293-26551	Limulus Test Tube-S	φ12 × 75 mm	10 pcs × 10
293-28251	Aluminum Cap-S	φ15 × 18 mm	10 pcs × 10
295-25151	Whole Pipette (endotoxin free)	5.2 mL (made of glass)	5 pcs

Endotoxin Measurement System

Toxinometer® ET-6000

Toxinometer® ET-6000 is a new computer-operated model, which is exceptionally user-friendly and expandable. Depending on the number of samples to process, extension modules can be connected, allowing endotoxin testing in a wide range of fields and sample quantities. The temperature setting can be switched between 30 and 37 ℃, facilitating (1 → 3)-β-D-glucan and peptidoglycan test for research projects.

■ Features

- Combined with the appropriate LAL reagent, three testing techniques: turbidimetric, chromogenic and gel-clot are usable with a single system.
- Simultaneous measurement of up to 16 samples is possible on a single module, which can be further enhanced to 512, when extension modules are connected (a total of 8 modules can be connected).
- Systems conforming to the FDA21 CFR Part 11 are also available.
- The software provided conforms to BET (USP/EP/JP).

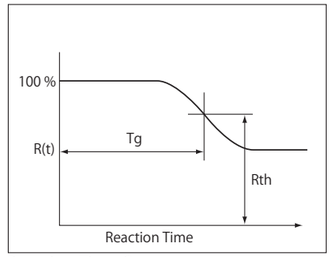
▲The maker or model name of the personal computer included in the system may be subject to change.

Code No.	Product Name	Power Source	Contents
Part 11 System			
293-33509	Toxinometer® ET-6000/U Part11 Set	100 – 120 ± 10 % VAC	Toxinometer® ET-6000 (1 unit) Toximaster® Part11 (5 licenses) Personal computer (1 unit) System validation documents
290-33519	Toxinometer® ET-6000/E Part11 Set	220 – 240 ± 10 % VAC	
Non Part 11 System			
293-33989	Toxinometer® ET-6000/U Non-Part11 Set	100 – 120 ± 10 % VAC	Toxinometer® ET-6000 (1 unit) Toximaster® (1 license) Personal computer (1 unit)
299-33969	Toxinometer® ET-6000/E Non-Part11 Set	220 – 240 ± 10 % VAC	
Expansion Module (Additional Module for Part11 Set or Non-Part11 Set)			
297-33529	Toxinometer® ET-6000/U	100 – 120 ± 10 % VAC	–
294-33539	Toxinometer® ET-6000/E	220 – 240 ± 10 % VAC	–
Option			
295-33461	Toxinometer® ET-6000 Rack	–	–

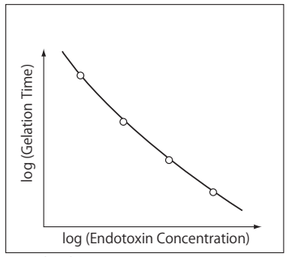
• Toxinometer® Measurement Principle

Toxinometer® translates the reaction of the LAL reagent with endotoxin into changes in the light intensity transmitted at 430 nm. The light intensity remains constant for a while after the start of reaction, but gradually decreases as the reaction proceeds, and stops upon completion of the reaction.
The ratio of the light intensity I(t) to the light intensity at an early stage of reaction Io is defined as R(t), and the threshold R(th) of R(t) is determined. Based on this, Toxinometer® reads the period from the start of the reaction to the point at which R(t) reaches R(th) as the gelation time Tg or the activation time Ta.

R(t) = [I(t)/Io] × 100 %



Reaction Time Course



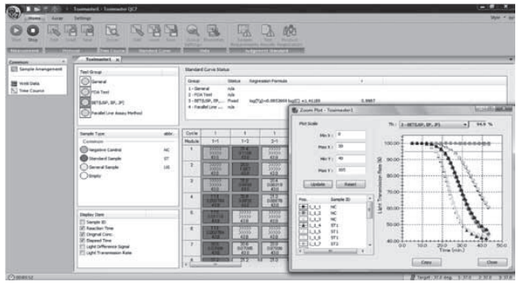
Standard Curve

• Quantification

A correlation between the endotoxin concentration and gelation time (Tg) is obtained, and the endotoxin concentration is calculated using the gelation time of a sample.

• Toximaster

Exclusive software for efficient routine work and high-quality data analysis



Endotoxin Measurement System

MPR Endotoxin Measurement System for BT

It is a system dedicated to endotoxin measurement using absorbance Microplate Reader ELx808IU and standard equipment"Toximaster® MPR" software. It can be used for various endotoxin tests such as quality control tests on pharmaceuticals and medical equipment. It enables you to arrange samples and input sample information on one software screen,and supports accurate multi-sample processing with simple and small software operation.

■ Features

- Combined with the appropriate LAL reagent, two testing techniques: turbidimetric and chromogenic are usable with a single system.
- Simultaneous measurement of up to 96 samples is possible with 96 well plate.
- Systems conforming to the FDA21 CFR Part 11 are also available.
- The software supports to BET (USP/EP/JP).
- Prepare dedicated software "Toximaster® MPR Part11" or "Toximaster® Non- Part11"