

Reagents and Related Products for Detection of Microbial Cell Components

Endotoxin

Peptidoglycan

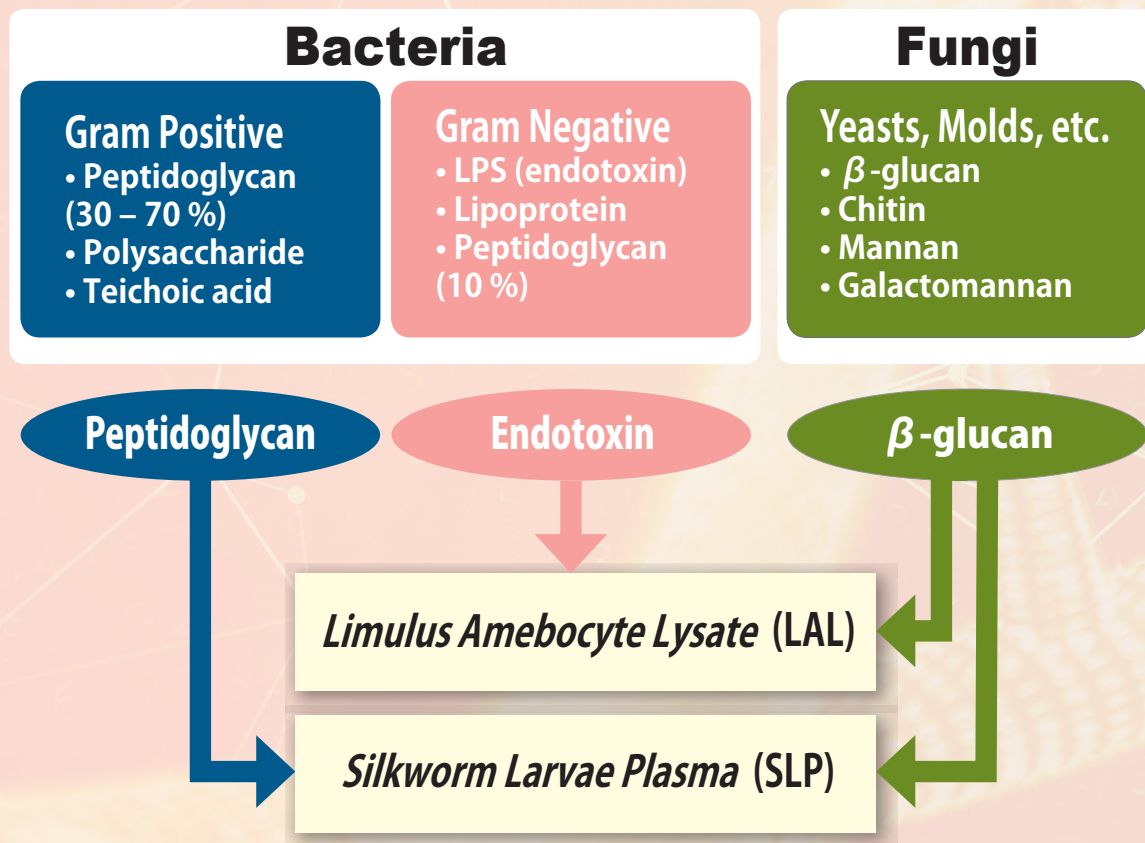
(1 → 3)- β -D-glucan



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Microbial Cell Wall Components



LAL Gel-clot Reagents

Bland Name	Type	Product Name	Gel-clot Sensitivity	Quantity	Page
PYROSTAR™	SINGLE TEST	PYROSTAR™ ES-F SINGLE TEST	0.015, 0.03 EU/mL	• 25 tests + 1 vial CSE (500ng/vial)	6~8p
	MULTI TEST	PYROSTAR™ ES-F MULTI KIT	0.015, 0.03, 0.06, 0.125, 0.25 EU/mL	• 80 tests + 1 vial CSE (500ng/vial) • 200 tests + 1 vial CSE (500ng/vial)	
		PYROSTAR™ ES-F MULTI KIT, BULK	0.015, 0.03, 0.06, 0.125, 0.25 EU/mL	• 2,000 tests • 5,000 tests	

LAL Turbidimetric Reagents

Bland Name	Type	Product Name	Quantitative Range	Quantity	Page
PYROSTAR™	SINGLE TEST For Toxinometer	PYROSTAR™ ES-F SINGLE TEST	0.001-10 EU/mL	• 25 tests + 1 vial CSE (500ng/vial)	6~8p
	MULTI TEST For Toxinometer	PYROSTAR™ ES-F MULTI KIT	0.001-10 EU/mL	• 80 tests+ 1 vial CSE (500ng/vial) • 200 tests+ 1 vial CSE (500ng/vial)	
		PYROSTAR™ ES-F MULTI KIT, BULK	0.001-10 EU/mL	• 2,000 tests • 5,000 tests	
	MULTI TEST For Microplate Reader	PYROSTAR™ ES-F/Plate with CSE	0.01-10 EU/mL	• 160 tests + 1 vial CSE (500ng/vial)	9p
		PYROSTAR™ ES-F/Plate without CSE	0.01-10 EU/mL	• 200 tests	

LAL Chromogenic Reagents

Bland Name	Type	Product Name	Quantitative Range	Quantity	Page
Color KY	SINGLE TEST For Toxinometer	Limulus Color KY Single Test wako	0.0002-5 EU/mL	• 25 tests + 1 vial CSE (500ng/vial)	10p
	MULTI TEST For Microplate Reader and Toxinometer	Limulus Color KY Test Wako	0.0005-5 EU/mL	• 60 tests* + 1 vial CSE (500ng/vial)	

※120 tests with microplate reader

SLP Reagent

Bland Name	Type	Product Name	Sensitivity	Quantity	Page
SLP	SINGLE TEST For Toxinometer	SLP-HS Single Reagents Set II	10 pg/mL (PG), 1 pg/mL (β -glucan)	• 20 tests + 2 vial (5.0mL) Diluent + 1 vial (0.5mL) Standard	11~12p

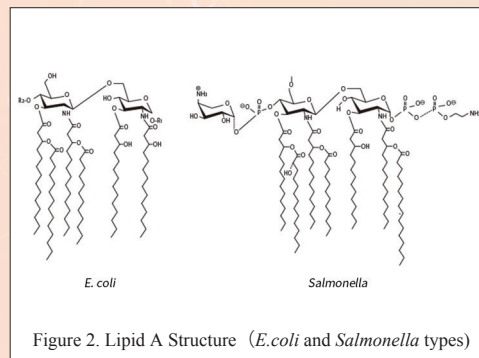
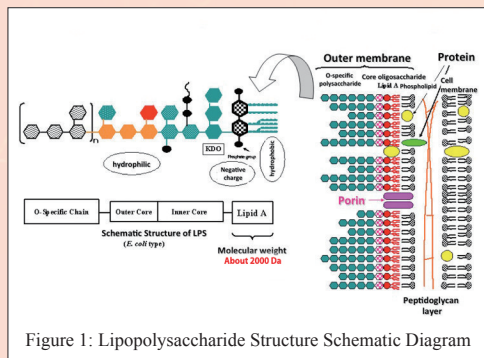
Basics of Bacterial Endotoxins Test

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.



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.

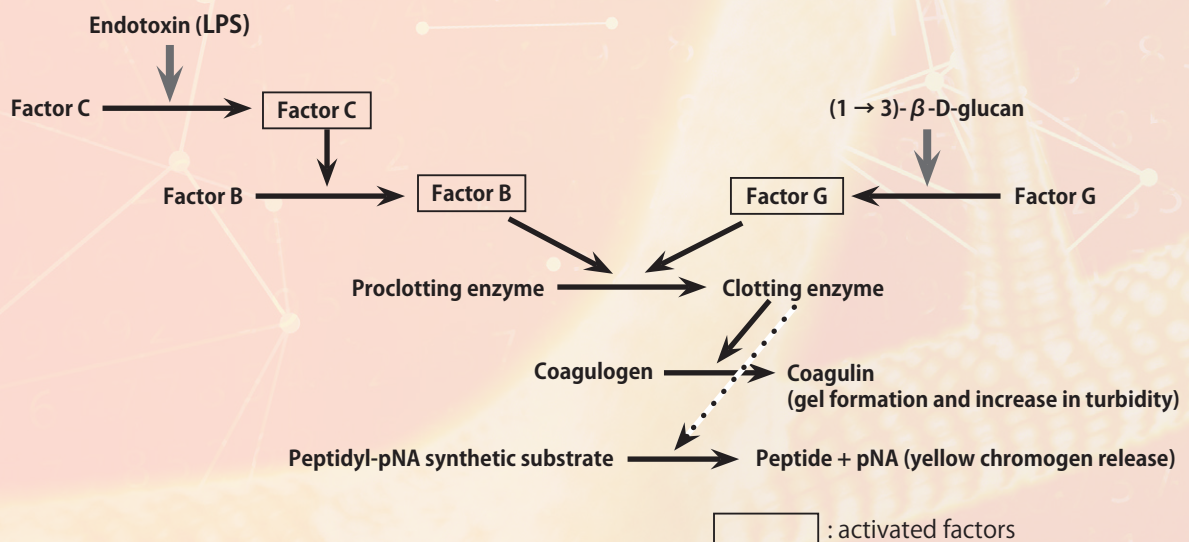


Figure 3: LAL Reagent Reaction Mechanism

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.

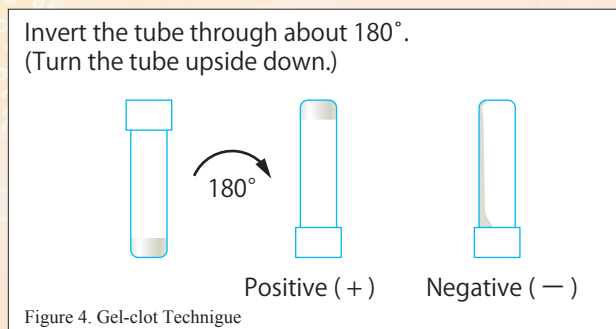
Various LAL reagents are commercially available, as well as measuring systems based on the Figure 3 reaction mechanism. It is essential to select the most appropriate product depending on the required accuracy, test frequencies, number of samples and other relevant factors.

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 $37 \pm 1^\circ\text{C}$, 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-F Series

Endotoxin-specific LAL reagents (not activated by (1→3)-β-D-glucan), compatible with the BET (USP) compliance tests U.S Food and Drug Administration (FDA) approved. They have various gelation sensitivities, 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

Kinetic turbidimetric measurement is available by combining the ES-F 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:

1 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

2 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)

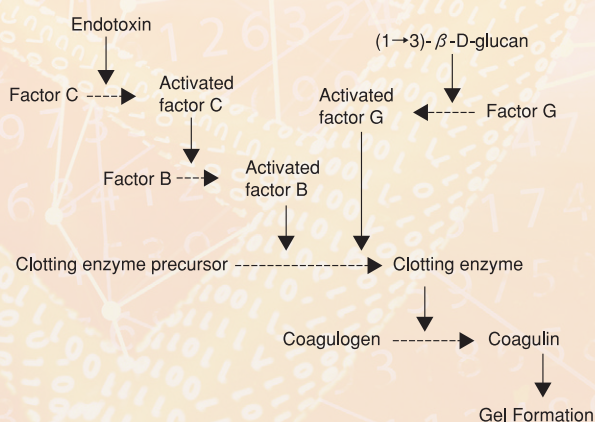
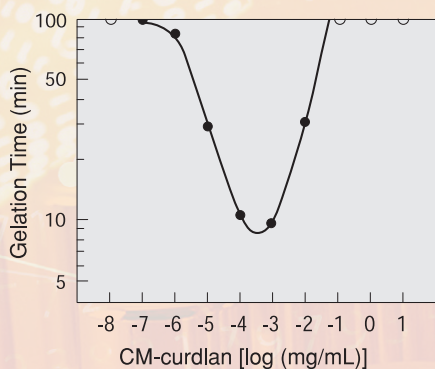


Figure 5: Limulus Test Reaction Cascade

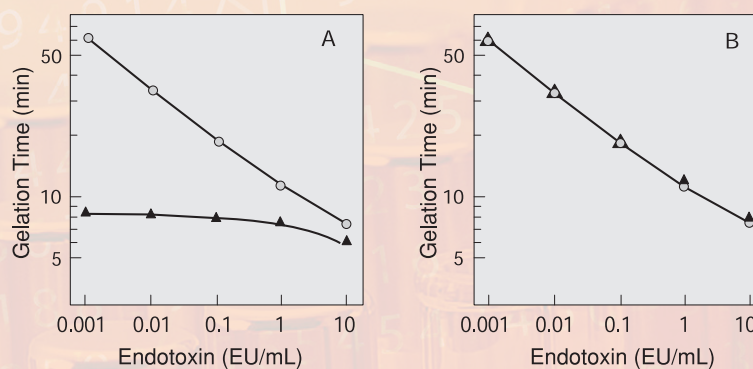
The reaction cascade mechanism of the LAL reagent and endotoxin is shown in Figure 5. If (1 → 3)-β-D-glucan* exists in the reaction system, it activates Factor G, causing a false positive reaction in which gelation occurs. This happens regardless of the presence of endotoxin, meaning endotoxin specific 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 curdian) 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 6: 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 7).

* (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 6: CM-curdian and LAL Reaction



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

Figure 7: Effect of CM-curdian on Endotoxin Measurement Using Limulus HS and Limulus ES

PYROSTAR™ ES-F Series

Turbidimetric Technique

Endotoxin Specific

FDA approved

Intended Use: Limulus amebocyte lysate (LAL) is intended for the detection of gram-negative bacterial endotoxins. PYROSTAR™ ES-F is intended for the qualitative detection of endotoxins by gel-clot or quantitative detection by kinetic turbidimetric methods.

- Endotoxin-specific lysate, avoids false positive results from glucans
- Available in multi-tests vials or single-test vials
- Can be used as either a gel-clot or Kinetic-Turbidimetric Assay (KTA) reagent
- (KTA) assays can be performed in tube reader or microplate reader
- Gel-Clot lysate sensitivities range from 0.015 to 0.25 EU/mL
- Available with matched control standard endotoxin (CSE)
- PYROSTAR™ ES-F reagents are available with a KTA quantitative range of either 0.001 EU/mL to 10 EU/mL.

The KTA quantitative range is related to the Gel-Clot sensitivity.

- 100uL sample size when used with tube reader; 50uL sample size when used with microplate reader

PYROSTAR™ ES-F Single Test Vial



Gel-clot Sensitivity (EU/mL)	KTA Quantitative Range (EU/mL)
0.015	0.001 to 10
0.03	0.01 to 10

Catalog Number	Product Name		Quantity
with CSE 295-72301	Limulus Amebocyte Lysate PYROSTAR™ ES-F SINGLE TEST 0.015		25 tests
with CSE 292-81601	Limulus Amebocyte Lysate PYROSTAR™ ES-F SINGLE TEST 0.03		25 tests

PYROSTAR™ ES-F (2 ML) Multi Test Vials with CSE



Gel-clot Sensitivity (EU/mL)	KTA Quantitative Range (EU/mL)
0.015	0.001 to 10
0.03 to 0.25	0.01 to 10

	Catalog Number	Code	Product Name	Quantity
with CSE	548-10141	WPEK4-20015	PYROSTAR™ ES-F MULTI KIT (2ML), 0.015 EU/ML	2mL x 4vials
with CSE	541-10131	WPEK4-20003	PYROSTAR™ ES-F MULTI KIT (2ML), 0.03 EU/ML	2mL x 4vials
with CSE	545-10151	WPEK4-20006	PYROSTAR™ ES-F MULTI KIT (2ML), 0.06 EU/ML	2mL x 4vials
with CSE	542-10161	WPEK4-20125	PYROSTAR™ ES-F MULTI KIT (2ML), 0.125 EU/ML	2mL x 4vials
with CSE	549-10171	WPEK4-20025	PYROSTAR™ ES-F MULTI KIT (2ML), 0.25 EU/ML	2mL x 4vials

CSE: 1 vial(500ng/vial)

PYROSTAR™ ES-F (2 ML) Multi Test Vials without CSE



Gel-clot Sensitivity (EU/mL)	KTA Quantitative Range (EU/mL)
0.015	0.001 to 10
0.03 to 0.25	0.01 to 10

Catalog Number	Code	Product Name	Quantity
546-10201	WPEM-20015	PYROSTAR™ ES-F MULTI KIT (2ML), BULK, 0.015 EU/ML	2mL x 100vials
546-10181	WPEM-20003	PYROSTAR™ ES-F MULTI KIT (2ML), BULK, 0.03 EU/ML	2mL x 100vials
543-10191	WPEM-20006	PYROSTAR™ ES-F MULTI KIT (2ML), BULK, 0.06 EU/ML	2mL x 100vials
540-10221	WPEM-20125	PYROSTAR™ ES-F MULTI KIT (2ML), BULK, 0.125 EU/ML	2mL x 100vials
543-10211	WPEM-20025	PYROSTAR™ ES-F MULTI KIT (2ML), BULK, 0.25 EU/ML	2mL x 100vials

PYROSTAR™ ES-F (5.2 ML) with CSE



Gel-clot Sensitivity (EU/mL)	KTA Quantitative Range (EU/mL)
0.015	0.001 to 10
0.03 to 0.25	0.01 to 10

	Catalog Number	Code	Product Name	Quantity
with CSE	543-10071	WPEK4-50015	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.015 EU/ML	5.2mL x 4vials
with CSE	542-10041	WPEK4-50003	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.03 EU/ML	5.2mL x 4vials
with CSE	547-10111	WPEK4-50006	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.06 EU/ML	5.2mL x 4vials
with CSE	544-10125	WPEK4-50125	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.125 EU/ML	5.2mL x 4vials
with CSE	549-10025	WPEK4-50025	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.25 EU/ML	5.2mL x 4vials

CSE: 1 vial(500ng/vial)

PYROSTAR™ ES-F (5.2 ML) without CSE



Gel-clot Sensitivity (EU/mL)	KTA Quantitative Range (EU/mL)
0.015	0.001 to 10
0.03 to 0.25	0.01 to 10

Catalog Number	Code	Product Name	Quantity
547-10231	WPEM-50015	PYROSTAR™ ES-F MULTI KIT (5.2ML), BULK, 0.015 EU/ML	5.2mL x 100vials
544-10241	WPEM-50003	PYROSTAR™ ES-F MULTI KIT (5.2ML), BULK, 0.03 EU/ML	5.2mL x 100vials
541-10251	WPEM-50006	PYROSTAR™ ES-F MULTI KIT (5.2ML), BULK, 0.06 EU/ML	5.2mL x 100vials
548-10261	WPEM-50125	PYROSTAR™ ES-F MULTI KIT (5.2ML), BULK, 0.125 EU/ML	5.2mL x 100vials
545-10271	WPEM-50025	PYROSTAR™ ES-F MULTI KIT (5.2ML), BULK, 0.25 EU/ML	5.2mL x 100vials

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.

Product 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 μ L 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			
Catalog Number	Product Name	Quantitative Range (EU/mL)	Quantity
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)

CSE: 1 vial(500ng/vial)

with CSE



The Limulus Color KY Series

Endotoxin Specific Limulus Reagent

Chromogenic Technique Endotoxin Specific

The Limulus Color KY Series includes both a multi-test kit and a single-test kit, each designed for time-based chromogenic analysis, using a synthetic substrate which produces a yellow color and can specifically detect endotoxin with high sensitivity.



Product features

- Endotoxin-specific lysate, avoids false positive results from glucans
- Available in multi-tests vials or single-test vials
- Quantitative Kinetic-Chromogenic Assay (KCA) reagent
- KCA assays can be performed in tube reader or microplate reader
- KCA quantitative range detection limit of 0.0002 EU/mL (single-type) and 0.0005 EU/mL (multi-type).
- Available with matched control standard endotoxin (CSE[®])
- 100uL sample size when used with tube reader; 50uL sample size when used with microplate reader

※CSE: 1 vial(500ng/vial)

Single-test

Catalog Number	Product Name	Quantity
291-53601	Limulus Color KY Single Test Wako	25 tests

CSE: 1 vial(500ng/vial)

Multi-test

Catalog Number	Product Name	Quantity
291-53101	Limulus Color KY Test Wako	60 tests

CSE: 1 vial(500ng/vial)

SLP-HS Single Reagent Set II 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 \rightarrow 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.

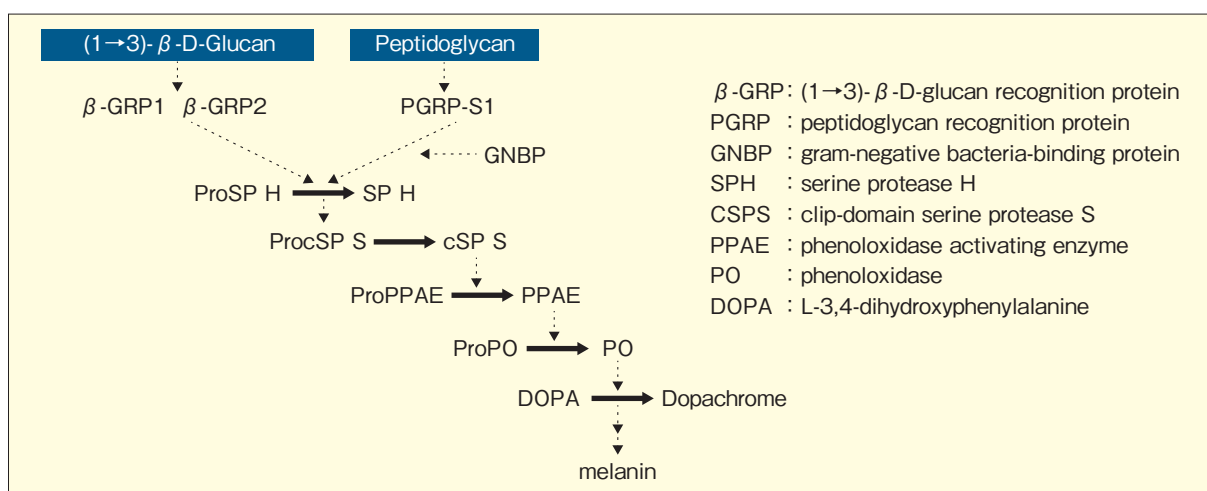


Figure 1. Phenoloxidase precursor cascade of *Bombyx mori* blood

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.

■ Product Features

- 1.High sensitive detection of PG and β -glucan
- 2.Accurate and sensitive quantification of PG and β -glucan with Toxinometer

■ Applications

- 1.Study of the structure-activity relationship, biosynthesis, metabolism and etiological significance of PG
- 2.Investigation of water pollution
- 3.Microbial contamination testing of the dialysate
- 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

- | | |
|---|----------------------|
| 1.SLP-HS Reagent II | for 0.1mL x 20 vials |
| (Lyophilized reagent containing <i>Silkworm Larvae</i> Plasma and DOPA) | |
| 2.SLP Diluent | for 5mL x 2 vials |
| 3.Standard | for 0.5mL x 1 vial |
| (Digested Peptidoglycan from <i>Staphylococcus aureus</i>) | |

■ Measurement Method

PG and β -glucan can be detected using SLP-HS Single Reagent Set and an instrument of Toxinometer. The measurement principle is the same with endotoxin detection method using Toxinometer (Figure 2). The absorbance change (melanin production) generated by the activation are detected using Toxinometer. The correlation between the PG(or β -glucan) concentration and T_a is obtained (Figure 3), and the PG (or β -glucan) concentration is calculated from T_a of each sample.

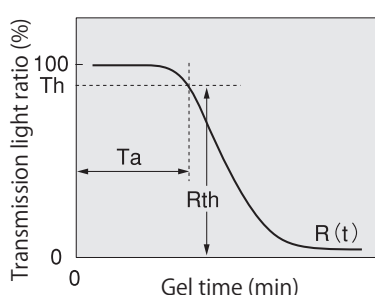


Figure 2. Measuring principles:
It measures time (T_a) of index moving from $R(t)$ to threshold (R_{th}). (Th : decision threshold)

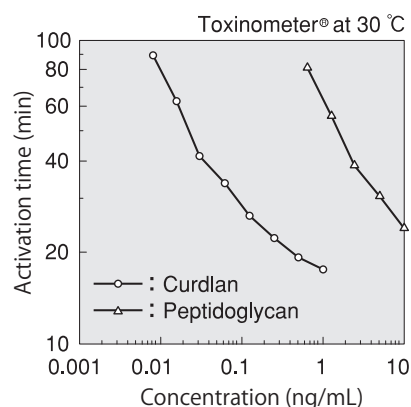


Figure 3. Measuring example using Toxinometer®

Catalog Number	Product Name	Quantity
296-81001	SLP-HS Single Reagent Set II	20 tests

Related Products		
Catalog Number	Product Name	Quantity
030-09903	Curdlan	1g
162-18101	Peptidoglycan, from <i>Micrococcus luteus</i>	2 mL

Toxinometer® ET-7000

The Toxinometer® ET-7000 is our computer-operated kinetic incubating tube reader, which is exceptionally user-friendly and easily expandable. Depending on the number of samples to be processed, our state of the art expansion modules can be connected to allow for endotoxin testing in a wide range of fields and sample quantities.

■ Product Features

- Kinetic Incubating Tube Reader
- Single-test configuration avoids “Hot Wells”
- For use in Kinetic-Turbidimetric, Kinetic-Chromogenic, and Gel-Clot assays
- A single assay module can simultaneously measure up to 16 samples
- Expansion modules are available to extend number of samples in multiples of 16
- Temperature settings at both 30°C and 37°C
- Endotoxin determination in compliance with FDA guidelines as well as with Pharmacopeal (USP/EP/JP) BET monographs



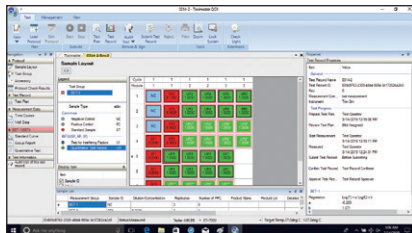
Catalog Number	Model	Power Source	Contents
Part 11 System ※The system complies with FDA21 CFR, Part 11.			
294-35871	Toxinometer® ET-7000/U Part11 Set	100-120 +/- 10% VAC (USA)	1 Toxinometer® ET-7000 Toximaster® QC8 Software w/5User Licenses
299-35821	Toxinometer® ET-7000/E Part11 Set	220-240 +/- 10% VAC (Europe)	1 Personal Computer System Validation Documents (in CD)
Non-Part 11 System ※The system doesn't comply with FDA21 CFR, Part 11.			
291-35881	Toxinometer® ET-7000/U Non-Part11 Set	100-120 +/- 10% VAC (USA)	1 Toxinometer® ET-7000 Toximaster® QC7 Software w/1User Licenses
296-35831	Toxinometer® ET-7000/E Non-Part11 Set	220-240 +/- 10% VAC (Europe)	1 Personal Computer
Expansion Module ※Additional Module for Part 11 Set or Non-Part 11 Set.			
297-35861	Toxinometer® ET-7000/U	100-120 +/- 10% VAC (USA)	16 well expansion unit
292-35811	Toxinometer® ET-7000/E	220-240 +/- 10% VAC (Europe)	

The "Welcome Screen" helps navigate you where you need to go!

Toximaster® Software

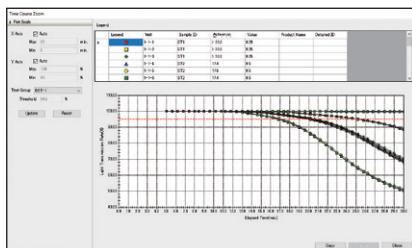
Exclusive software for Efficient routine work & High quality analysis.

Protocol Settings

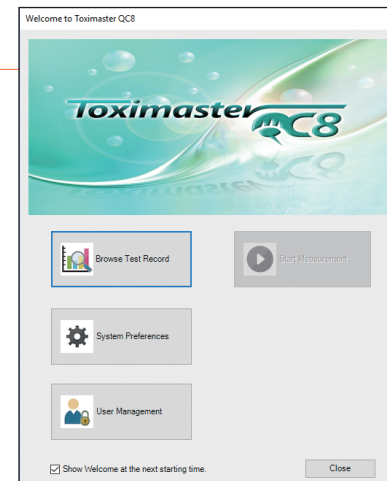


Easy to edit! Once you create a protocol, you can start a measurement immediately.

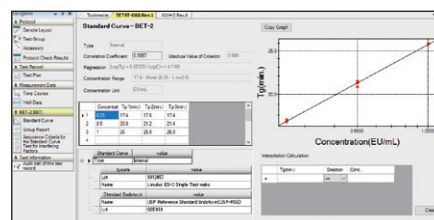
Time Course Graph



Enables visual confirmation of measurement status. You can predict results and prepare the next steps.



Standard Curve



Conveniently monitored! All information can be seen on one screen.

Part 11 Functions ※Ensure data integrity

Electronic Signature

Login

FUJIFILM Wako Pure Chemical Corporation

Account Name:

Password:

All measurement records are linked to signatures. Never allows for manipulation and falsification.

Audit Trail

#	Id	Comment	Operation	Time	Time	Time	Time	Time	Time
1	210701010101	CLL1000-01	Test	2011/07/01 10:01:01	2011/07/01 10:01:01	2011/07/01 10:01:01	2011/07/01 10:01:01	2011/07/01 10:01:01	2011/07/01 10:01:01
2	210701010102	CLL1000-02	Test	2011/07/01 10:01:02	2011/07/01 10:01:02	2011/07/01 10:01:02	2011/07/01 10:01:02	2011/07/01 10:01:02	2011/07/01 10:01:02
3	210701010103	CLL1000-03	Test	2011/07/01 10:01:03	2011/07/01 10:01:03	2011/07/01 10:01:03	2011/07/01 10:01:03	2011/07/01 10:01:03	2011/07/01 10:01:03
4	210701010104	CLL1000-04	Test	2011/07/01 10:01:04	2011/07/01 10:01:04	2011/07/01 10:01:04	2011/07/01 10:01:04	2011/07/01 10:01:04	2011/07/01 10:01:04
5	210701010105	CLL1000-05	Test	2011/07/01 10:01:05	2011/07/01 10:01:05	2011/07/01 10:01:05	2011/07/01 10:01:05	2011/07/01 10:01:05	2011/07/01 10:01:05
6	210701010106	CLL1000-06	Test	2011/07/01 10:01:06	2011/07/01 10:01:06	2011/07/01 10:01:06	2011/07/01 10:01:06	2011/07/01 10:01:06	2011/07/01 10:01:06
7	210701010107	CLL1000-07	Test	2011/07/01 10:01:07	2011/07/01 10:01:07	2011/07/01 10:01:07	2011/07/01 10:01:07	2011/07/01 10:01:07	2011/07/01 10:01:07
8	210701010108	CLL1000-08	Test	2011/07/01 10:01:08	2011/07/01 10:01:08	2011/07/01 10:01:08	2011/07/01 10:01:08	2011/07/01 10:01:08	2011/07/01 10:01:08
9	210701010109	CLL1000-09	Test	2011/07/01 10:01:09	2011/07/01 10:01:09	2011/07/01 10:01:09	2011/07/01 10:01:09	2011/07/01 10:01:09	2011/07/01 10:01:09
10	210701010110	CLL1000-10	Test	2011/07/01 10:01:10	2011/07/01 10:01:10	2011/07/01 10:01:10	2011/07/01 10:01:10	2011/07/01 10:01:10	2011/07/01 10:01:10
11	210701010111	CLL1000-11	Test	2011/07/01 10:01:11	2011/07/01 10:01:11	2011/07/01 10:01:11	2011/07/01 10:01:11	2011/07/01 10:01:11	2011/07/01 10:01:11
12	210701010112	CLL1000-12	Test	2011/07/01 10:01:12	2011/07/01 10:01:12	2011/07/01 10:01:12	2011/07/01 10:01:12	2011/07/01 10:01:12	2011/07/01 10:01:12
13	210701010113	CLL1000-13	Test	2011/07/01 10:01:13	2011/07/01 10:01:13	2011/07/01 10:01:13	2011/07/01 10:01:13	2011/07/01 10:01:13	2011/07/01 10:01:13
14	210701010114	CLL1000-14	Test	2011/07/01 10:01:14	2011/07/01 10:01:14	2011/07/01 10:01:14	2011/07/01 10:01:14	2011/07/01 10:01:14	2011/07/01 10:01:14
15	210701010115	CLL1000-15	Test	2011/07/01 10:01:15	2011/07/01 10:01:15	2011/07/01 10:01:15	2011/07/01 10:01:15	2011/07/01 10:01:15	2011/07/01 10:01:15
16	210701010116	CLL1000-16	Test	2011/07/01 10:01:16	2011/07/01 10:01:16	2011/07/01 10:01:16	2011/07/01 10:01:16	2011/07/01 10:01:16	2011/07/01 10:01:16
17	210701010117	CLL1000-17	Test	2011/07/01 10:01:17	2011/07/01 10:01:17	2011/07/01 10:01:17	2011/07/01 10:01:17	2011/07/01 10:01:17	2011/07/01 10:01:17
18	210701010118	CLL1000-18	Test	2011/07/01 10:01:18	2011/07/01 10:01:18	2011/07/01 10:01:18	2011/07/01 10:01:18	2011/07/01 10:01:18	2011/07/01 10:01:18
19	210701010119	CLL1000-19	Test	2011/07/01 10:01:19	2011/07/01 10:01:19	2011/07/01 10:01:19	2011/07/01 10:01:19	2011/07/01 10:01:19	2011/07/01 10:01:19
20	210701010120	CLL1000-20	Test	2011/07/01 10:01:20	2011/07/01 10:01:20	2011/07/01 10:01:20	2011/07/01 10:01:20	2011/07/01 10:01:20	2011/07/01 10:01:20

Major procedures are recorded automatically. History of operation can be confirmed as a log file.

Operation Authorities

Each account belongs to a specific group and each group can define its own authorities for operation.

- Modify System Preferences
- Register Instrument
- Register Protocol
- Register Reagent
- Register Accessory
- Register Standard Curve Data
- Register Product
- Load Protocol into Test Record
- Star Measurement
- Load Test Record
- Review Test Plan
- Confirm Test Record
- Approve Test Record
- Submit Test Record

Etc ..

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.

■ Product 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 comply with the FDA21 CFR Part 11 are also available.
- The software supports to BET (USP/EP/JP).
- Prepare dedicated software "Toximaster® MPR Part11" or "Toximaster® MPR Non- Part11"



Catalog Number	Product Name	Contents
Part 11 system		
292-35931	Toximaster® QC8 MPR Part 11 PC Set	Toximaster® QC8 MPR Part 11 Software Personal Computer System Validation Document(in CD)
Non-Part 11 system		
-	Toximaster® QC7 MPR Non-Part 11 PC Set	Toximaster® QC7 MPR Non-Part 11 Software Personal Computer

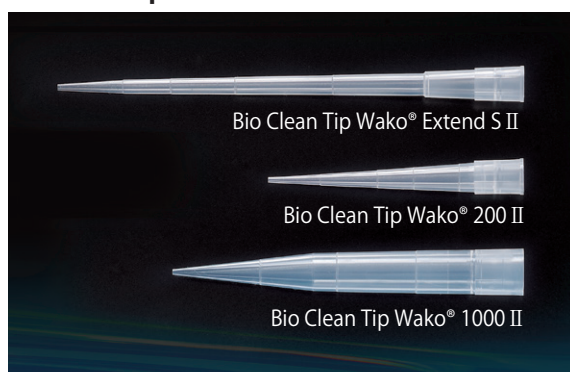
Endotoxin Test Related Accessories

BioClean® Series

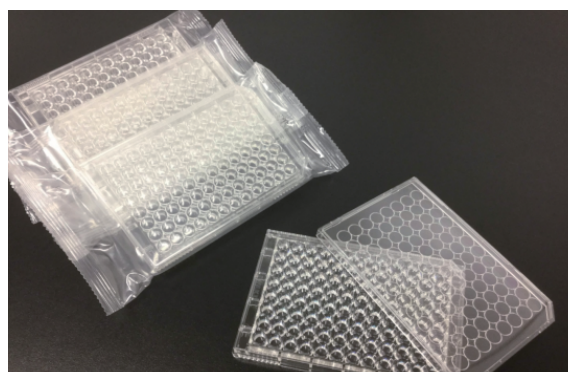
■ Product Features

- Endotoxin-free pipette tip (<0.005 EU/tip)
- Individually packaged, ideal for use in clean rooms
- Packaging film features dust-free protection
- Sterilized by gamma irradiation (25kg ray)
- Tips fit Finnpiquette digital micro pipettors (20-200uL or 100-1000uL sizes)
- Endotoxin-free tubes and caps sterilized at 250 °C
- For use with Toxinometer® measurement system

Bio Clean Tip Wako®



Bio Clean Plate Wako®



Endotoxin-free Pipette Tips and Microplates			
Catalog Number	Product Name	Volume Size	Quantity
294-35011	BioClean Tip Wako® Extend S II	200 μ L	100 tips
291-35021	BioClean Tip Wako® 200 II	200 μ L	100 tips
298-35031	BioClean Tip Wako® 1000 II	1000 μ L	100 tips
293-35221	Bio Clean Plate Wako® Microplate	96 well	50 pcs/pk

Limulus Test Tube-S with Aluminum Cap



Endotoxin-free Test Tubes and Caps/ Gel-clot Reaction Tubes			
Catalog Number	Product Name	Volume Size	Quantity
292-32751	Limulus Test Tube-S with Aluminum Cap	12 x 75mm	80 pcs
293-26551	Limulus Test Tube-S	12 x 75mm	100 pcs
293-28251	Aluminum Cap-S	14.7 x 18mm	100 pcs

Gel-clot Reaction Tubes

■ Product Features

- Endotoxin-free test tubes (<0.001 EU/tube)
- Designed for Gel-clot testing
- Made with Borosilicate glass

Made with borosilicate glass



Catalog Number	Product Name	Volume Size	Quantity
CT-1075	Gel-clot Reaction Tube	10×75mm	200 tubes/pk
CT-1075B	Gel-clot Reaction tube,Bulk	10×75mm	1250 tubes/pk
542-10281	Depyrogenated Dilution Tube	13 x 100mm	50 pcs/pk

Endotoxin Test Related Accessories

Control Standard Endotoxin

■ Product Features

- Endotoxin derived from *E. Coli* UKT-B
- Can be used to prepare controls and standard curves
- RSE/CSE ratios supplied to match specific lots of LAL
- Reconstituted CSE can be stored at 2-10°C for 1 month



Catalog Number	Code	Product Name	Quantity
638-01021	-	Japanese Pharmacopoeia Endotoxin Reference Standard	1 vial (10,000~25,000 EU/vials)
546-10061	CSE 4037-5006	Control Standard Endotoxin	6 vials (500 ng/vial)

LAL Reagent Water

Wako proudly provides high-quality, endotoxin free water ideal for all of your LAL testing needs.

■ Product Features

- Endotoxin-free water (<0.001 EU/mL)
- Steam sterilized by USP standards
- Derived from water for injection, USP
- Non LAL reactive



Catalog Number	Code	Product Name	Quantity
540-10081	LRW-12100	Lysate Reagent Water, 100mL	12 x 100mL vials
547-10091	LRW-2030	Lysate Reagent Water, 30mL	20 x 30mL vials

Endotoxin Extracting Solution

Endotoxin Extracting Solution for LAL Test

Traditionally, water or saline solution has been used to extract endotoxin in tests on medical devices and equipment; however, the efficacy of this extraction method has recently come into question. In order to provide our customers with a more reliable method for medical device testing, FUJIFILM Wako has developed an endotoxin-free extracting solution containing human serum albumin (HSA) that is capable of extracting endotoxins which cannot be extracted in water or saline solution.

■ Product Features

- Capable of extracting endotoxins from surfaces which cannot be extracted in water or saline
- Recommended for use in endotoxin testing on equipment and devices which may come into contact with fluids containing blood or protein.



Catalog Number	Product Name	Quantity
293-51601	Endotoxin Extracting Solution for LAL Test	4 x 10 mL vials

Endotoxin Indicator

Endotoxin Indicator Vial (>1,000 EU/vial)

■ Product Features

Intended Use: The Endotoxin indicator is intended for performing validation for depyrogenation processes of dry heat oven cycles. To ensure that a cycle is effectively inactivating and/or destroying endotoxin, an analysis comparing the endotoxin concentration before and after the depyrogenation process is completed. The United States Pharmacopeia (USP) describes preparing endotoxin indicators of a sufficient concentration to allow recovery of a minimum 1,000 Endotoxin Units (EU) in order to accurately demonstrate at least a 3-log reduction in endotoxin levels during the depyrogenation process challenge. The Endotoxin indicator is intended to demonstrate a minimum 3-log reduction after depyrogenation when used with PYROSTAR™ ES-F series by gel clot and kinetic methods



Catalog Number	Code	Product Name	Quantity
549-10291	EIV-025	Endotoxin Indicators, >1,000 EU/vial	25 vials

Contribution to Measurement Technology - Acquisition of FDA approval

Endotoxin is a potent toxin contained in cell wall components of Gram-negative bacteria. Since it brings about the fever or lethal shock when entering the blood of a person, it is necessary to confirm that medicines are not contaminated with endotoxin. We have strived to evolve the endotoxin measurement technology by manufacturing lysate reagents and developing the measurement system "Toxinometer", and supported medicines from behind for over 30 years.

From sales of lysate reagents to the development of endotoxin measurement system



Our US Plant (Location: Richmond, Virginia)

measurement reagent (Limulus reagent, renamed as lysate reagent later) prepared from the blood extract of horseshoe crabs and "Gel-clot technique" using this were developed. Gel-clot technique were listed in the US Pharmacopoeia in 1980, but we also began importing and selling the reagent prior to this. However, in the gel-clot technique, problems remained such as being the semi-quantitative method and the judgment accuracy depending on the proficiency of the measurer.

Initially, the rabbit pyrogen test was used for the endotoxin test method. After injecting the sample into the rabbit, the amount of endotoxin contamination was measured from the increasing degree of its body temperature. However, with this test method, there were drawbacks such as taking time to get results, being unable to maintain the reliable results because of using animals.

In 1956 in the United States, F.B.Bang discovered a phenomenon that the blood of horseshoe crabs solidified in a gel state with endotoxin, and then the endotoxin

We challenged to solve these problems, then in 1985, developed the measurement method "Turbidimetric time analysis method" based on the different principle from the conventional one and released its measurement system "Toxinometer" for the first time in the world. At the same time, in the United States, ACC company (Associates of Cape Cod, .INC) developed a system using the same measurement principle. In response to this situation, the turbidimetric time analysis method was listed in the US FDA Guideline in 1987.

Currently, Toxinometers corresponding to the turbidimetric, chromogenic and gel-clot technique are provided along with numerous reagents. Meanwhile, also offering "MPR Endotoxin Measurement System" corresponding to the chromogenic and turbidimetric techniques using plate readers, we are contributing to the safety of pharmaceutical production and quality control of pharmaceuticals as a pioneer of endotoxin measurement.

Establishment of the FDA approved plant and development of the world's only specific reagent for endotoxin

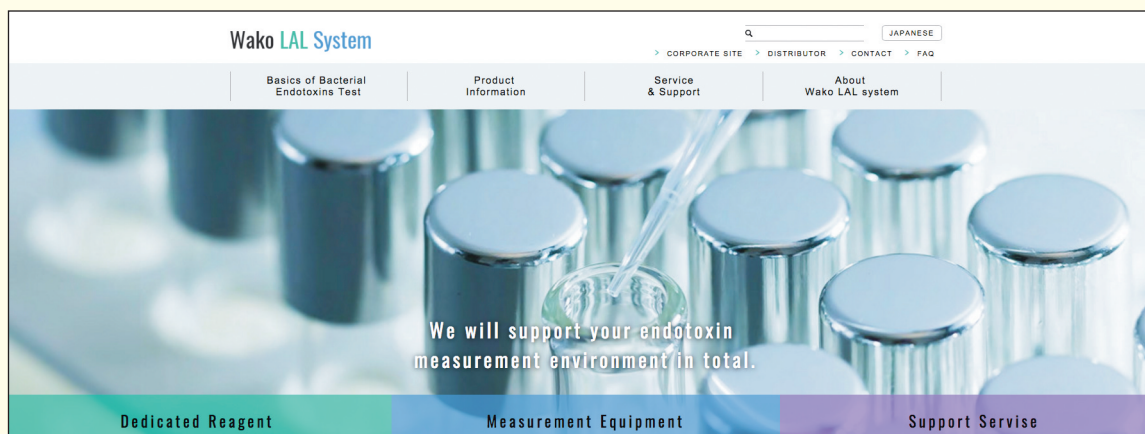
The world's first lysate reagent was developed in the United States, and we initially handled only imported items from the United States. As the market expanded and the needs of reagents increased, we established a plant in the United States. First of all, we bought Hemakem Inc. in St. Louis in 2003 and made it a foothold for entering the United States. After that, we built a new plant in Richmond, received FDA approval, and began shipping reagents. Incidentally, the endotoxin specific reagents with FDA approval are only our products worldwide.

Also, there is a convenient single type reagent that is lyophilized in a test tube beforehand and can be measured simply by putting the sample. Even in this category, the company having the endotoxin specific reagents with FDA approval is only us in the world.

As described above, our company, which has been involved in endotoxin reagents for more than 30 years and making efforts on the improvement of measurement methods and the development of "Toxinometer", has tried hard to develop the pharmaceutical industry and others, as one of pioneers of endotoxin measurement.

Wako LAL System

Web Site for Endotoxin Analysis



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

Contact

<http://ffwk.fujifilm.co.jp/en/contact/index.html>

FUJIFILM Wako Pure Chemical Corporation

<http://ffwk.fujifilm.co.jp/>
1-2,Doshomachi 3-Chome
Chuo-Ku,Osaka 540-8605,Japan

FUJIFILM Wako Chemicals U.S.A. Corporation

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