

LAL and SLP Reagents and Related Products for Detection of Microbial Cell Wall Components



CONTENTS

Basics of Bacterial Endotoxins Test	3
Endotoxin-Specific LAL Reagents	
<hr/>	
PYROSTAR™ ES-F Single Test	9
PYROSTAR™ ES-F MULTI KIT (2 ML) with CSE	10
PYROSTAR™ ES-F MULTI KIT (2 ML) BULK without CSE	10
PYROSTAR™ ES-F MULTI KIT (5.2 ML) with CSE	11
PYROSTAR™ ES-F MULTI KIT (5.2 ML) BULK without CSE	11
PYROSTAR™ ES-F/Plate	12
Limulus Color KY Series	13
Recombinant Cascade Reagent	
<hr/>	
PYROSTAR™ Neo +	14
Peptidoglycan and β-D-Glucan Detection Reagent	
<hr/>	
SLP-HS Single Reagent Set II	15
Endotoxin analysis systems	
<hr/>	
Toxinometer® ET-7000	18
Microplate Reader EPOCH2	19
Toximaster®FQC1	20
Endotoxin test related accessories	
<hr/>	
BioClean Series	22
Endotoxin-free Test Tubes and Caps/ Gel-clot Reaction Tubes	22
Gel-clot Reaction Tubes	22
Control Standard Endotoxin	23
LAL Reagent Water	23
Endotoxin Test Related Products	
<hr/>	
Endotoxin Extracting Solution for LAL Test	24
ES-Buffer	24
LPS for endotoxin indicator	24

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.

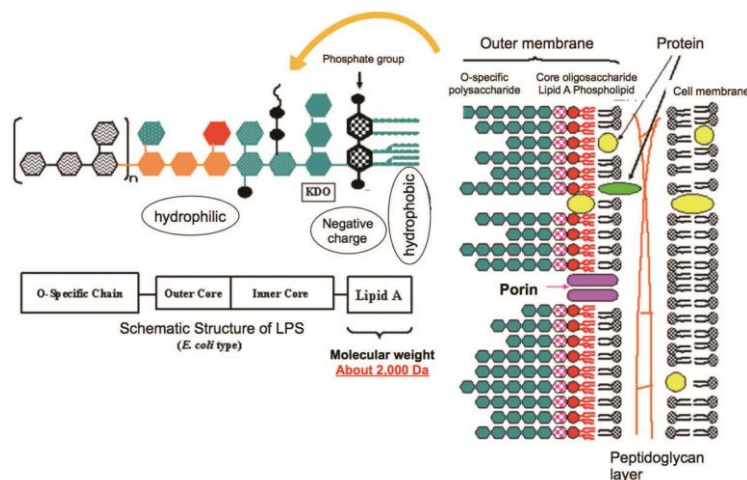


Figure 1 : Lipopolysaccharide Structure Schematic Diagram

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 2, 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.

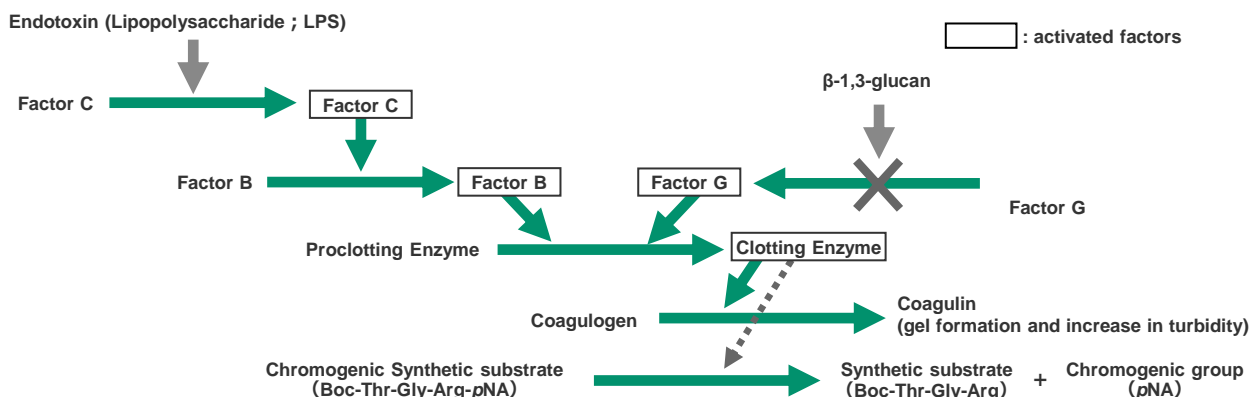


Figure 2 : LAL Reagent Reaction Mechanism

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.

The β-glucan pathway inhibitors are co-lyophilized in our LAL reagents, allowing endotoxin-specific measurements without any special process.

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.

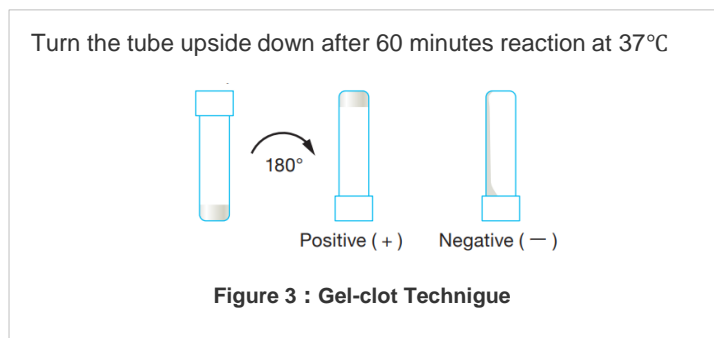
Pharmacopoeias in the U.S., Europe and Japan refer to three means of endotoxin detection, namely the gel-clot technique, chromogenic and turbidimetric techniques, which are detailed in the following sections while introducing the characteristics and application examples of our relevant LAL reagents

(1) Turbidimetric technique and gel-clot technique

These methods detect the gelation reaction of LAL reagents by endotoxin, and there are the turbidimetric technique that optically detects the change in turbidity due to the gelation reaction and the gel-clot technique that qualitatively visually determines gel formation.

The turbidimetric technique requires an optical detector such as a Toxinometer® or Microplate reader, but quantitative measurement is possible.

The gel-clot technique has the advantage of not requiring a device, but it is a qualitative measurement that determines whether the reagent is positive or negative based on the gelation sensitivity of the reagent. If you use a Toxinometer®, you can easily perform quantitative measurement by the nephelometer analysis method using the same procedure as the gel-clot technique.



> Types of Reagents

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, especially in quantitative measurement using Toxinometer®. The multi-type kit is ideal 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

The ES-F series are 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) licensed.

They have various gelation sensitivities, and are available in both single- and multi- type kits. 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.

*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

> 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.

> 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. Consumables 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.

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).

3 Influence on Signal detection

Gel-clot technique

- Vibration during incubation
- Inappropriate judgment technique

Optical method

- Bubbles
- Foreign matter and solids
- Coloring and turbidity

6. Principle of Limulus ES (Endotoxin Specific Reagent)

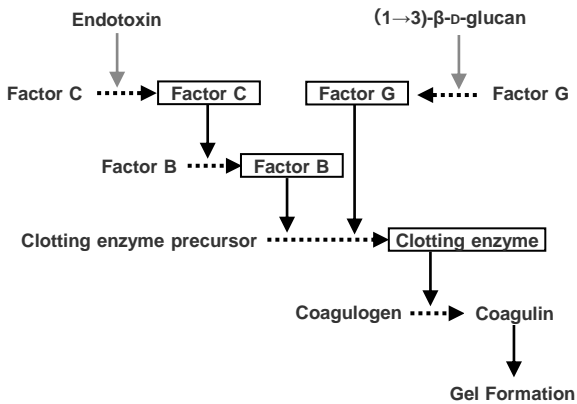


Figure 4 : Limulus Test Reaction Cascade

The reaction cascade mechanism of the LAL reagent and endotoxin is shown in Figure 4. 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(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 5: 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 6).

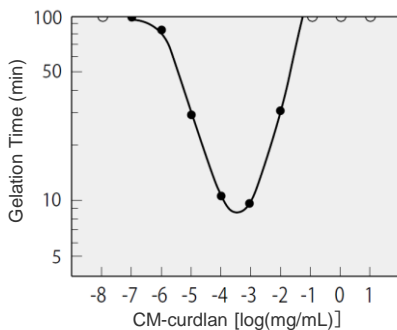


Figure 5 : CM-curdlan and LAL Reaction

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

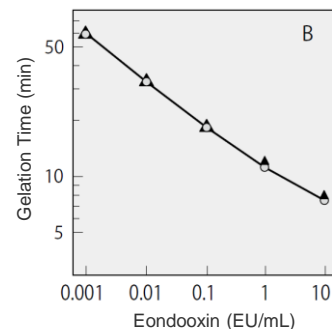
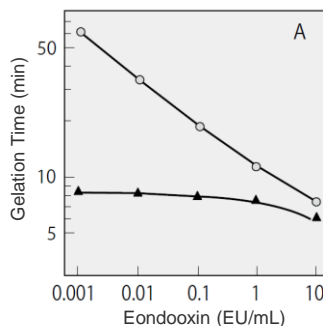
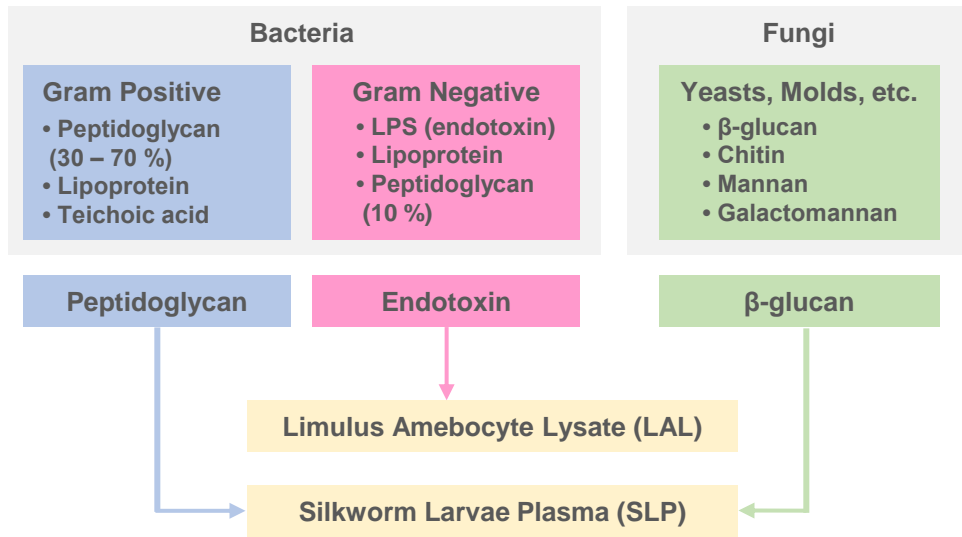


Figure 6 : Effect of CM-curdlan on Endotoxin Measurement Using Limulus HS and Limulus ES

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

Microbial Cell Wall Components



Choosing which reagent is right for your testing

1 Gel-clot

Bland Name	Type	Measurement system	Product Name	Gel-clot Sensitivity	Quantity	Page
PYROSTAR™	SINGLE TEST	Toxinometer®	PYROSTAR™ ES-F SINGLE TEST	0.015, 0.03, 0.125, 0.25 EU/mL	25 tests + 1 vial CSE (500ng/vial)	9
			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)	10-11
	MULTI TEST		PYROSTAR™	0.015, 0.03, 0.06, 0.125, 0.25 EU/mL	• 2,000 tests	10-11
			PYROSTAR™ ES-F MULTI KIT, BULK	0.125, 0.25 EU/mL	• 5,000 tests	

2 Turbidimetric

Bland Name	Type	Measurement system	Product Name	Quantitative Range	Quantity	Page	
PYROSTAR™	SINGLE TEST	Toxinometer®	PYROSTAR™ ES-F SINGLE TEST	0.001-10 EU/mL	25 tests + 1 vial CSE (500ng/vial)	9	
			PYROSTAR™ ES-F MULTI KIT	0.001-10 EU/mL	• 80 tests + 1 vial CSE (500ng/vial) • 200 tests + 1 vial CSE (500ng/vial)	10-11	
	MULTI TEST		PYROSTAR™ ES-F MULTI KIT, BULK	0.001-10 EU/mL	• 2,000 tests • 5,000 tests	10-11	
			Microplate Reader	PYROSTAR™ ES-F/Plate	0.01-10 EU/mL	• 160 tests + 1 vial CSE (500ng/vial) • 400 tests + 1 vial CSE (500 ng/vial)	12

3 Chromogenic

Bland Name	Type	Measurement system	Product Name	Quantitative Range	Quantity	Page
Color KY	SINGLE TEST	Toxinometer®	Limulus Color KY Single Test wako	0.0002-5 EU/mL	25 tests + 1 vial CSE (500ng/vial)	13
	MULTI TEST	Toxinometer®, Microplate Reader	Limulus Color KY Test Wako	0.0005-5 EU/mL	60 tests* + 1 vial CSE (500ng/vial)	13

4 Recombinant Cascade Reagent

Bland Name	Type	Measurement system	Product Name	Quantitative Range	Quantity	Page
PYROSTAR™	MULTI TEST	Microplate Reader	PYROSTAR™ Neo+	0.001-50 EU/mL	50 tests	14

5 Peptidoglycan, β-glucan

Bland Name	Type	Measurement system	Product Name	Sensitivity	Quantity	Page
SLP	SINGLE TEST	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	15



Endotoxin-Specific LAL Reagents

Endotoxin-Specific LAL Reagents

PYROSTAR™ ES-F Series

FEATURES

- 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

■ PYROSTAR™ ES-F Single Test

Single Test	Turbidimetric Technique	Gel-clot Technique	Endotoxin Specific	FDA licensed
Catalog No.	Manufacturer Code No.	Product Name	CSE	Quantity
540-10101	WPESK-0015	PYROSTAR™ ES-F Single Test Kit, 0.015EU/mL	✓	25 tests
547-10351	WPESK-0003	PYROSTAR™ ES-F Single Test Kit, 0.03EU/mL	✓	25 tests
—	WPESK-0006	PYROSTAR™ ES-F Single Test Kit, 0.06EU/mL	✓	25 tests
541-10371	WPESK-0125	PYROSTAR™ ES-F Single Test Kit, 0.125EU/mL	✓	25 tests
544-10361	WPESK-0025	PYROSTAR™ ES-F Single Test Kit, 0.25EU/mL	✓	25 tests



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

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

Multi Test	Turbidimetric Technique	Gel-clot Technique	Endotoxin Specific	FDA licensed
------------	-------------------------	--------------------	--------------------	--------------

Catalog No.	Manufacturer Code No.	Product Name	CSE	Quantity
548-10141	WPEK4-20015	PYROSTAR™ ES-F MULTI KIT (2ML), 0.015 EU/ML	✓	2mL x 4vials
541-10131	WPEK4-20003	PYROSTAR™ ES-F MULTI KIT (2ML), 0.03 EU/ML	✓	2mL x 4vials
545-10151	WPEK4-20006	PYROSTAR™ ES-F MULTI KIT (2ML), 0.06 EU/ML	✓	2mL x 4vials
542-10161	WPEK4-20125	PYROSTAR™ ES-F MULTI KIT (2ML), 0.125 EU/ML	✓	2mL x 4vials
549-10171	WPEK4-20025	PYROSTAR™ ES-F MULTI KIT (2ML), 0.25 EU/ML	✓	2mL x 4vials



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

■ PYROSTAR™ ES-F (2 ML) Multi Test , Bulk without CSE

Multi Test	Turbidimetric Technique	Gel-clot Technique	Endotoxin Specific	FDA licensed
------------	-------------------------	--------------------	--------------------	--------------

Catalog No.	Manufacturer Code No.	Product Name	CSE	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



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

■ PYROSTAR™ ES-F (5.2 ML) Multi Test with CSE

Multi Test	Turbidimetric Technique	Gel-clot Technique	Endotoxin Specific	FDA licensed
------------	-------------------------	--------------------	--------------------	--------------

Catalog No.	Manufacturer Code No.	Product Name	CSE	Quantity
543-10071	WPEK4-50015	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.015 EU/ML	✓	5.2mL x 4vials
542-10041	WPEK4-50003	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.03 EU/ML	✓	5.2mL x 4vials
547-10111	WPEK4-50006	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.06 EU/ML	✓	5.2mL x 4vials
544-10121	WPEK4-50125	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.125 EU/ML	✓	5.2mL x 4vials
549-10051	WPEK4-50025	PYROSTAR™ ES-F MULTI KIT (5.2ML), 0.25 EU/ML	✓	5.2mL x 4vials



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

■ PYROSTAR™ ES-F (5.2 ML) Multi Test, Bulk without CSE

Multi Test	Turbidimetric Technique	Gel-clot Technique	Endotoxin Specific	FDA licensed
------------	-------------------------	--------------------	--------------------	--------------

Catalog No.	Manufacturer Code No.	Product Name	CSE	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



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

PYROSTAR™ ES-F/Plate

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) licensed.
- 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	Turbidimetric Technique	Endotoxin Specific	FDA licensed
------------	-------------------------	--------------------	--------------

Catalog No.	Manufacturer Code No.	Product Name	Quantitative Range (EU/mL)	CSE	Quantity
543-10331	WPEPK4-20015	PYROSTAR™ ES-F/PLATE TEST 2 mL x 4 vials	0.01 to 10	✓	160 tests (2mL x 4vials)
540-10341	WPEPK4-50015	PYROSTAR™ ES-F/PLATE TEST 5.2 mL x 4 vials	0.01 to 10	✓	400 tests (5.2mL x 4vials)
548-10401	WPEPM-20015	PYROSTAR™ ES-F/PLATE BULK KIT 2mL x 100 vials	0.01 to 10		4000 tests (2mL x 100vials)
—	WPEPM-50015	PYROSTAR™ ES-F/PLATE BULK KIT 5.2mL x 100vials	0.01 to 10		10,000 tests (5.2mL x 100vials)



Endotoxin-Specific LAL Reagents Limulus Color KY Series

Chromogenic Technique (Kinetic Yellow Synthetic Substrate Method)

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
- 200uL sample size for KY Single Test with)

Single Test	Chromogenic Technique	Endotoxin Specific			
Catalog No.	Product Name	Quantitative Range (EU/mL)	CSE	Quantity	
291-53601	Limulus Color KY Single Test Wako	0.0002 to 5	✓	25 tests	CSE : 1 vial(500ng/vial)

Multi test	Chromogenic Technique	Endotoxin Specific			
Catalog No.	Product Name	Quantitative Range (EU/mL)	CSE	Quantity	
291-53101	Limulus Color KY Test Wako	0.0005 to 5	✓	60 tests *	CSE : 1 vial(500ng/vial) * 120 tests with Microplate reader



PYROSTAR™ Neo+ (Coming soon)

FEATURES

- Since colorimetric method, the same colorimetric plate reader can be used.
- Same cascade reaction as Lysate reagent, conforms to traditional detection.
- Endotoxin specific reagent, no false positive risk of (1→3)-β-D-glucan.
- Quantification of endotoxin with a wide range of 0.001 to 50 EU / mL and high sensitivity
- Could be stored at 2-8°C about 4H after solution/at -30 degree for about 2 weeks. Reagent waste is reduced.

Catalog No.	Product Name	Quantitative Range (EU/mL)	Quantity
293-36941	PYROSTAR™ Neo+	0.001-50	50 tests (2.7mL x 1vial)

Principle

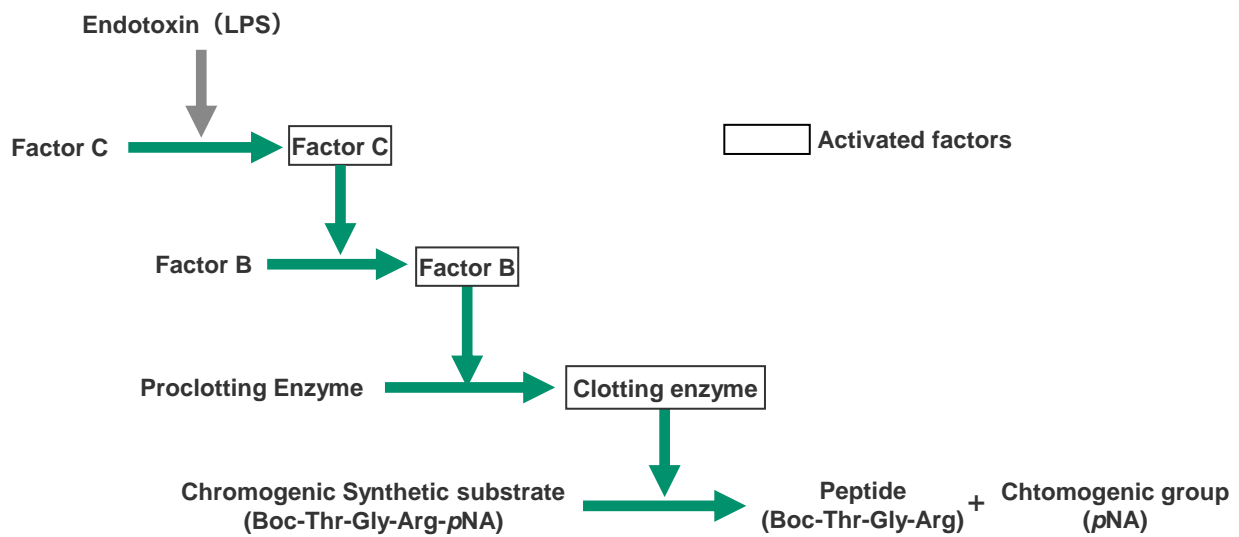


Figure1. PYROSTAR™ Neo+ Reaction Mechanism

Peptidoglycan and β -D-Glucan Detection Reagent SLP-HS Single Reagent Set II

Silkworm Larvae Plasma Reagent

FEATURES

- High sensitive detection of PG and β -glucan
- Accurate and sensitive quantification of PG and β -glucan with Toxinometer®

Single Test	Peptidoglycan	β -D-Glucan
Catalog No.	Product Name	Quantity
296-81001	SLP-HS Single Reagent Set II	20 tests

Related Products

Catalog No.	Product Name	Quantity
030-09903	Curdlan	1g

Kit Contents

項目	容量
SLP-HS (Silkworm Larvae Plasma High Sensitive) Reagent II	0.1mL x 20vials
SLP-Diluent	5mL x 2vials
Standard (Digested Peptidoglycan from <i>S. aureus</i>)	0.5mL x 1vial

Principle

The hemolymph of the silkworm *Bombyx mori* contains a self-defense mechanism termed the "prophenoloxidase (pro PO) activating system" or "pro PO cascade".

Upon invasion of microbe such as bacteria and fungi, the system participates in melanin formation observed in the body fluid of insects to protect them from the invader's attack.

The system is triggered by peptidoglycan (PG) from bacteria and (1 \rightarrow 3)- β -D-glucan (β -glucan) from fungi and yeast, and consequently, pro PO in the system is activated.

The system is thought to be a cascade reaction involving activation of multiple zymogens of proteinases.

The SLP (Silkworm Larvae Plasma) reagent is a lyophilized product containing factors of the proPO cascade, it is prepared from silkworm larvae body fluid and processed without melanin formation. The SLP reagent is activated by PG and/or β -glucan then L-3,4-dihydroxyphenylalanine (DOPA) is oxidized and melanin is formed. Since β -glucan is found in cell walls of many fungi, it is possible to detect various microorganisms by using melanin pigment formation of SLP reagent as an index.

The activation mechanism of SLP is considered as shown in Figure 1. Either PG or β -glucan binds to the respective recognition proteins termed PGRP or β -glucan binds to the respective recognition proteins termed PGRP or β GRP.

The proPO cascade system is initiated by these reactions, and consequently, proPO is converted to phenoloxidase (PO). The PO catalyzes oxidation of DOPA and is followed by the formation of melanin in the mixture. In the kinetic colorimetric assay with the Toxinometer, the amount of melanin pigment by SLP reaction is monitored as a change in the light transmission rate, and the reaction time until the light transmission rate reaches a preset threshold value is defined as activation time (T_a).

SLP activating substances (PG and β -glucan) are quantified with T_a as an index.

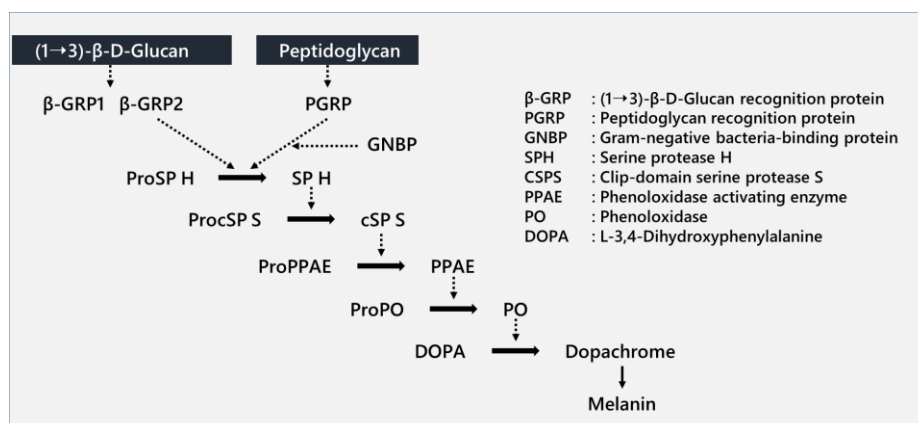


Figure 1. Phenoloxidase precursor cascade of *Bombyx mori* blood

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

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 Ta is obtained (Figure 3), and the PG (or β -glucan) concentration is calculated from Ta of each sample.

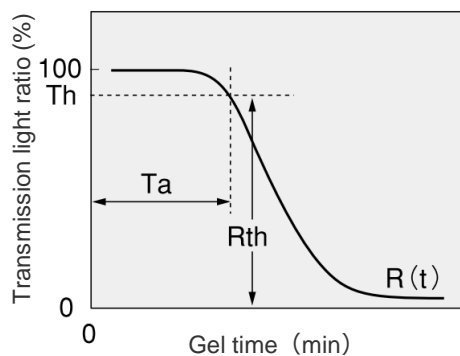


Figure 2. Measuring principles:
It measures time (Ta) of index moving from R(t) to threshold (Rth). (Th: decision threshold)

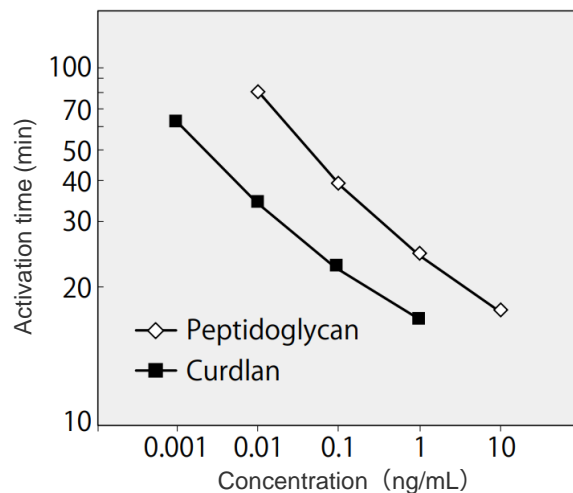


Figure 3. Measuring example using Toxinometer[®]





Endotoxin analysis systems

Toxinometer® ET-7000

FEATURES

- Kinetic Incubating Tube Reader
- Single-test configuration avoids “Hot Wells”
- For use in Kinetic-Turbidimetric, Kinetic-Chromogenic, and Ge I-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 No.	Product Name	Contents
293-36061	Toxinometer® ET-7000	1 Toxinometer® ET-7000

■ Related Products

Item	Catalog No.	Product Name	Contents
Software	290-36831	Toximaster® FQC1 PC Set E	<ul style="list-style-type: none"> • 1 Personal computer • Toximaster® FQC1 Software • System Validation Doc.
Accessories	295-36761	Toxinometer® 240V power cord*	<ul style="list-style-type: none"> • Toxinometer® 240V power cord (1unit)

* It's required when using in the 200~240 V area.

■ Specification

Item	Explanation	
Functions	Transmitted light quantity measuring function (capable of measuring 16 samples simultaneously) Temperature control function Automatic light quantity check function	
Light source	High intensity blue LED Central wavelength: 430 nm	
Detector	Silicon photocell	
Temperature control	Dry bath: 30±1.0 degrees C/37±1.0 degrees C (can be changed by software for ET-7000) Warmup time: 20 minutes (when preset temperature is 37 degrees C and surrounding temperature is 25 degrees C)	
Display	The LED indicates measurement The LED indicates errors and information during checking	
Weight	6.3 kg (±10%)	
Size	W 190 mm × D 420 mm × H 130 mm (protrusions not included)	
Power source	AC100-240 V (±10%)	
Frequency	50/60 Hz	
Power consumption	Max 120 W	
Environment	During operation When temperature is set at 37 degrees C Ambient temperature: 15 to 30 degrees C Humidity: 30 to 85%, non-condensing When temperature is controlled at 30 degrees C Ambient temperature: 15 to 25 degrees C Humidity: 30 to 85%, non-condensing	
	During stored	Ambient temperature: -20 to 60 degrees C Humidity: 30 to 85%, non-condensing
	Location	Indoor
	Altitude	2000 m or lower

EPOCH2

FEATURES

- 96 well configuration, Ideal for high throughput of samples
- Fast kinetics, endpoint and linear well scanning
- Extensive endotoxin analysis with Toximeter[®]FQC1 software



Manufacturer Code No.	Product Name	Contents
EPOCH2NS	EPOCH2 Microplate Spectrophotometer	1 EPOCH2 unit

■ Related Products

Item	Catalog No.	Product Name	Contents
Software	290-36831	Toximeter [®] FQC1 PC Set E	<ul style="list-style-type: none"> • 1 Personal computer • Toximeter[®] FQC1 Software • System Validation Doc.

■ Specification

Item	Explanation
Size	W 320 mm × D 393 mm × H 203 mm
Weight	11.3 kg
Temperature control	Dry bath: 30±1.0 degrees C/37±1.0 degrees C (can be changed by software for ET-7000) Warmup time: 20 minutes (when preset temperature is 37 degrees C and surrounding temperature is 25 degrees C)
Display	Xenon flashlamp

Toximaster® FQC1

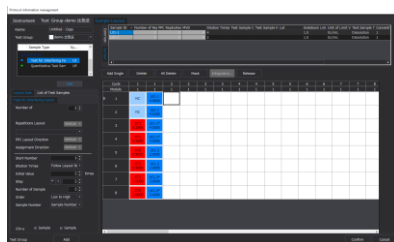
FEATURES

- Endotoxin determination in compliance with FDA guidelines as well as with Pharmacopeal (USP/EP/JP) BET monographs
- Capable of creating three types of curves: internal standard curve, manual input curve and a measured curve
- Allows trending of product results over time and early detection of potential product failures
- Creation of a standard workflow to be reviewed, confirmed and approved prior to operating
- Security functions to lock the application, disable an account and lock out the system



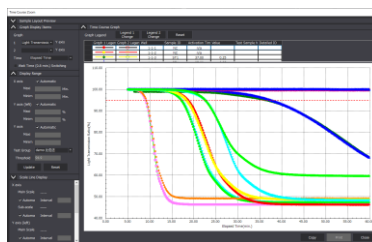
Catalog No.	Product Name	Contents
290-36831	Toximaster® FQC1 PC Set E	<ul style="list-style-type: none"> • 1 Personal computer • Toximaster® FQC1 Software • System Validation Doc

■ Protocol Settings



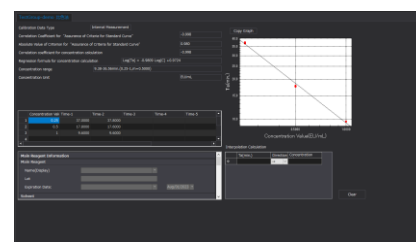
Easy to edit !
Once you create a protocol, you can start a measurement immediately.

■ Time Course Graph



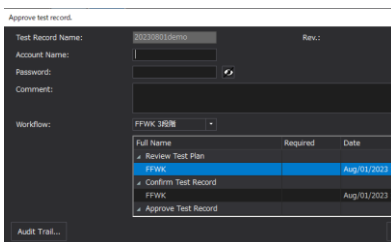
Enables visual confirmation of measurement status.
You can predict results and prepare the next steps.

■ Standard Curve



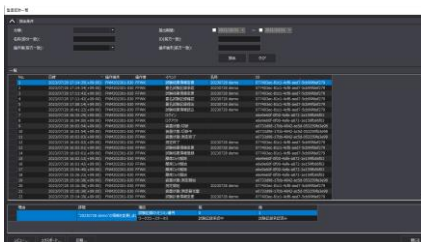
Conveniently monitored!
All information can be seen on one screen

■ Electronic Signature



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

■ Audit trail



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

■ Operation Authorities

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



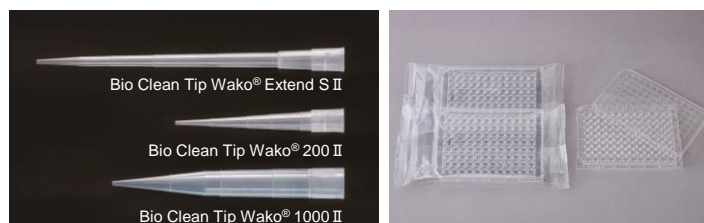
Endotoxin test related accessories
Endotoxin Test Related Products

Endotoxin test related accessories

BioClean Series

FEATURES

- Endotoxin-free pipette tip
- Individually packaged, ideal for use in clean rooms
- Packaging film features dust-free protection



■ Bio Clean Tip Wako®

Code No.	Product Name	Volume Size	Quantity
294-35011	BioClean Tip Wako® Extend S II	200µL	100 tips
291-35021	BioClean Tip Wako® 200 II	200µL	100 tips
298-35031	BioClean Tip Wako® 1000 II	1000µL	100 tips

■ Bio Clean Plate Wako™

Catalog No.	Product Name	Quantity
293-35221	BioClean Plate Wako™ (Microplate)	50 pcs/pk

Endotoxin-free Test Tubes and Caps

FEATURES

- Endotoxin-free



Catalog No.	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 *1	15 x 18mm	100 pcs
290-13751	Aluminium Cap-M *2	10.5 x 11.3mm	100 pcs

* 1 : for 13 x 100mm and 12 x 75mm tubes * 2 : for 10 x 75mm tubes

Gel-clot Reaction Tubes

FEATURES

- Endotoxin-free
- Designed for Gel-clot testing
- Made with Borosilicate glass



Catalog No.	Manufacture Code No.	Product Name	Volume Size	Quantity
542-10301	CT-1075	Gel-clot Reaction Tube	10 x 75mm	200 tubes/pk
549-10311	CT-1075B	Gel-clot Reaction tube, BULK	10 x 75mm	1,250 tubes/pk
542-10281	DL-13100	Depyrogenated Dilution Tube	13 x 100mm	50 pcs/pk

Control Standard Endotoxin

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 No.	Manufacture Code No.	Product Name	Quantity
546-10061	CSE 4037-5006	Control Standard Endotoxin	6vials (500ng/vial)

LAL Reagent Water

FEATURES

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



Catalog No.	Manufacture Code No.	Product Name	Quantity
547-10091	LRW-2030	LAL Reagent Water, 30 mL	20 x 30mL glass bottles
546-10321	LRW-12125	LAL Reagent Water, 125 mL	12 x 125mL plastic bottles

Endotoxin Test Related Products

Endotoxin Extracting Solution for LAL Test

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.



Code No.	Product Name	Quantity
293-51601	Endotoxin Extracting Solution for LAL Test	4 x 10mL vials

ES-Buffer

FEATURES

- Each vial has solution for use in reconstituting a max of 5.2 mL of reagent
- Ideal for use in reconstituting any LAL that has the potential to react with glucans, thus making an endotoxin-specific reagent



Code No.	Manufacturer Code No.	Product Name	Quantity
548-10381	ESB-0006	ES Buffer	6 x 6mL vials

LPS for endotoxin indicator

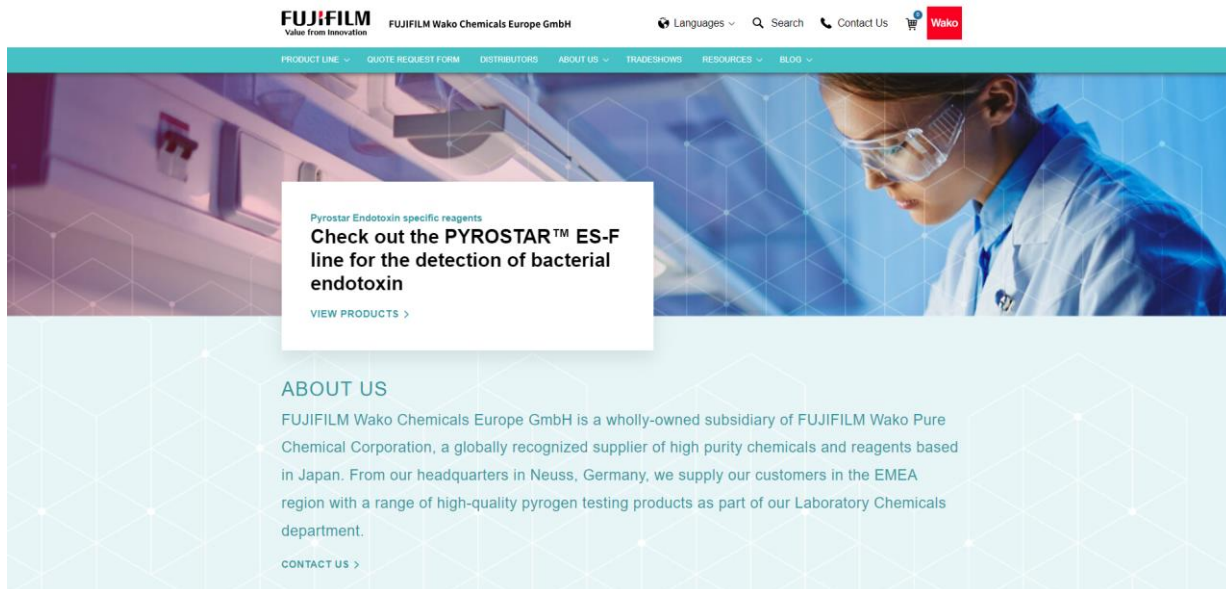
FEATURES

- E. coli 055:B5 (> 100,000 EU/vial)
- Can make multiple endotoxin indicators from one vial
- Used for validation studies



Code No.	Manufacturer Code No.	Product Name	Quantity
545-10391	WLPS-0100K	LPS for Endotoxin Indicator	6 vials (>100,000 EU/vial)

Wako Pyrogen Web Site for Endotoxin Analysis



FUJIFILM
Value from innovation

FUJIFILM Wako Chemicals Europe GmbH

Languages Search Contact Us Wako

PRODUCT LINE QUOTE REQUEST FORM DISTRIBUTORS ABOUT US TRADESHOWS RESOURCES BLOG

Pyrostar Endotoxin specific reagents
Check out the PYROSTAR™ ES-F line for the detection of bacterial endotoxin
VIEW PRODUCTS >

ABOUT US
FUJIFILM Wako Chemicals Europe GmbH is a wholly-owned subsidiary of FUJIFILM Wako Pure Chemical Corporation, a globally recognized supplier of high purity chemicals and reagents based in Japan. From our headquarters in Neuss, Germany, we supply our customers in the EMEA region with a range of high-quality pyrogen testing products as part of our Laboratory Chemicals department.
CONTACT US >

Contact

<https://www.wako-pyrogen.com/contact-us/>

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use. / Please visit FUJIFILM Wako Laboratory Chemicals site: <https://labchem-wako.fujifilm.com/> / This leaflet may contain products that cannot be exported to your country due to regulations. / Bulk quote requests for some products are welcomed. Please contact us.

FUJIFILM Wako Laboratory Chemicals site **<https://labchem-wako.fujifilm.com>**

1-2, Doshomachi 3-Chome, Chuo-ku, Osaka 540-8605, Japan
Tel: +81 6 6203 3741 Fax: +81 6 6203 1999
ffwk-cservise@fujifilm.com

FUJIFILM Wako Chemicals U.S.A. Corporation
1600 Bellwood Road, Richmond, VA 23237, U.S.A.
Toll-Free (U.S. only): +1 877 714 1920
Tel: +1 804 271 7677 Fax: +1 804 271 7791
wkuslabchem@fujifilm.com

FUJIFILM Wako Chemicals (Hong Kong) Limited
Room 1111, 11/F, International Trade Centre, 11-19 Sha Tsui Road,
Tsuen Wan, N.T., Hong Kong
Tel: +852-2799-9019 Fax: +852-2799-9808
wkhk.info@fujifilm.com

FUJIFILM Wako Chemicals Europe GmbH
Fuggerstr 12, 41468 Neuss, Germany
Tel: +49 2131 311 0 Fax: +49 2131 311 100
labchem_wkeu@fujifilm.com

FUJIFILM Wako (Guangzhou) Trading Corporation
Room 3003, 30/F., Dong Shan Plaza 69, Xian Lie Zhong Road,
Guangzhou, 510095, China
Tel: +86-20-8732-6381(Guangzhou) Tel: +86-21-6288-4751(Shanghai)
Tel: +86-10-6413-6388(Beijing)
wkgz.info@fujifilm.com