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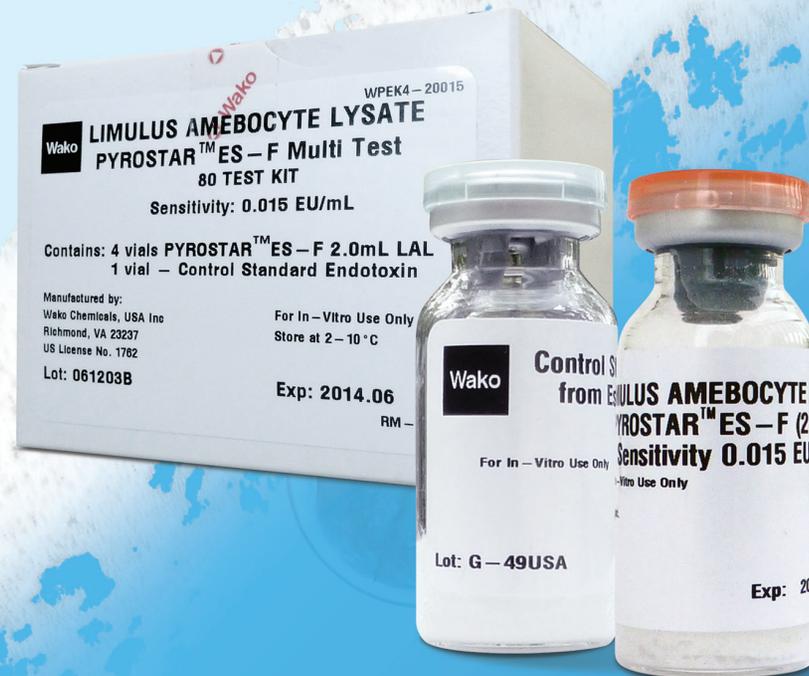


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PYROSTAR™

LAL Reagent Products for Detection of
BACTERIAL ENDOTOXIN



The PYROSTAR™ ES-F Series

Wako

Wako Chemicals USA, Inc.

Unique Endotoxin-Specific LAL Reagents that combine the simplicity of the Gel-Clot method with the quantitation of a Kinetic Turbidimetric Assay

Why Use An Endotoxin-Specific LAL Reagent?

Untreated LAL reagents react not only with endotoxin, but also with β (1 \rightarrow 3)-D-glucan, a fungal cell wall component that initiates the clotting cascade by activating the Factor G pathway. Glucan activation results in false positive endotoxin results! To eliminate this β (1 \rightarrow 3) -D-glucan activation, various endotoxin-specific reagents have been developed by either removing Factor G or inhibiting its activation.

The PYROSTAR™ ES-F series of reagents represent WAKO's uniquely endotoxin specific line of Limulus Amebocyte Lysate products, which are formulated to be used either qualitatively as a gel-clot reagent or quantitatively as a kinetic-turbidimetric reagent.



PYROSTAR™ ES-F Series

The PYROSTAR™ ES-F series of reagents are endotoxin-specific and unreactive to β (1-3)-D-glucan. In addition, these products are formulated to be used as either a gel-clot reagent or a kinetic-turbidimetric reagent.

The reagents in this series are available in either a single-test format with reaction vials already containing pre-dispensed LAL reagent for a single measurement, or a multi-test format requiring an aliquot from the dissolved reagent to be dispensed into depyrogenated glass test tubes. The single-test format is ideal for an assay with a few samples, and the multi-test format for a larger number of samples.

The multi-test reagent 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. The single-test reagent vial is used by adding 0.2 mL of the sample directly to the reaction vial with pre-dispensed, lyophilized LAL reagent.

1) Gel-Clot Assay

Gel-clot LAL reagents are supplied with a specified lysate sensitivity or lambda (λ). Lysate sensitivity is the minimum concentration of endotoxin required to generate a gel-clot. Samples, standards or controls are mixed with the LAL reagent in a depyrogenated glass test tube and incubated undisturbed at 37 ± 1 °C for 60 ± 2 minutes. Upon completion of the incubation period the tubes are slowly inverted through 180°. If a gel has formed and maintains its integrity without deformation or collapse throughout the 180° inversion, the result is scored a positive, while it is scored a negative if no gel has formed and the reaction mixture remains a liquid. Typically, a series of sample dilutions (usually 2-fold) are assayed. The highest positive dilution is referred to as the endpoint dilution. The endotoxin concentration in the sample is calculated by multiplying the denominator of the endpoint dilution by the lysate sensitivity.

Example:

Lysate sensitivity = 0.03 EU / mL

Endpoint Dilution = 1 / 10

Endotoxin Concentration = 10×0.03 EU / mL = 0.3 EU / mL

The PYROSTAR™ ES-F series is available with lysate sensitivities ranging from 0.015 to 0.25 EU/mL, and are available in both single and multi-test vials.

(2) Kinetic Turbidimetric Assay (KTA)

The kinetic turbidimetric assay uses the rate of change in reagent turbidity to quantitate the amount of endotoxin in samples. The onset of turbidity is referred to as the Tg. Tg is typically measured in minutes.

The Tg is inversely related to the endotoxin concentration. The higher the endotoxin concentration, the faster the reaction mixture becomes turbid and the shorter the Tg. The lower the endotoxin concentration, the slower the reaction mixture becomes turbid the longer the Tg.

Kinetic Turbidimetric LAL reagents are supplied with a quantitative range of endotoxin concentrations over which the amount of endotoxin in a sample can be quantitated. The Tg of standards containing a known amount of endotoxin is used to construct a standard curve relating Tg to its corresponding endotoxin concentration. The endotoxin concentration in samples and controls is calculated from their respective Tg's and the standard curve.

The Kinetic Turbidimetric Assay can be performed in either depyrogenated glass test tubes or plastic microtiter plates.

An incubating kinetic tube reader and associated software (see Toxinometer) must be used when performing the KTA in glass test.

An incubating kinetic microplate reader and associated software must be used when performing the KTA in plastic microplates.

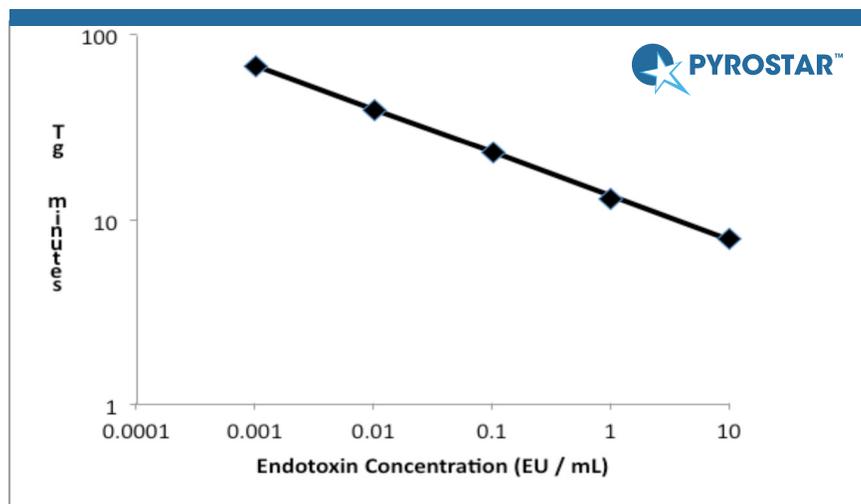
Calculation of endotoxin concentration

During a KTA reaction the turbidity of the test solutions is continually monitored by the Toxinometer. The time required for a sample to reach a determined absorbance level over background is measured. This time is referred to by the Toxinometer software as Tg.

The software produces a log(x axis)/log(y axis) correlation of the Tg of each standard with its corresponding endotoxin concentration. An example of a standard series and recovery of an endotoxin spike of 1.0 EU/mL in product is presented below:

Representative Standard Curve

Standard	EU/mL	Tg (minutes)
1	0.001	68.8
2	0.01	40.0
3	0.1	23.6
4	1.0	13.2
5	10	8.0
slope	-0.235	
Y intercept	1.13	
correlation	-0.999	



PYROSTAR™ ES-F reagents are available with a KTA quantitative range of either 0.001 EU / mL to 10 EU / mL or 0.01 EU / mL to 10 EU / mL. The KTA quantitative range is related to the gel-clot sensitivity.

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

Product Features

- Avoids false positive results
 - Endotoxin-specific lysate, avoids false positive results from glucans
 - Single-test (Tube) configuration avoids “Hot Wells”
- Available in multi-tests vials or single-test vials
- Can be used as either a gel-clot or KTA (Kinetic-Turbidimetric Assay) 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 or 0.01 EU/mL to 10 EU/mL. The KTA quantitative range is related to the Gel-Clot sensitivity.

PYROSTAR™ ES-F PLATE

Our same reliable endotoxin specific formulation for use with microplate readers.

Product Features

- Endotoxin specific testing
- Quantitative detection by kinetic turbidimetric method
- KTA Quantitative Range (EU/mL) 0.01 to 10
- For use with microplate readers
- Formulated for no interference from beta-Glucans
- No buffer required to block beta-Glucan interference

Multi-Test Lysate with matched CSE

PYROSTAR™ ES-F 2.0 mL Test Kit • 4 multi-test vials (2.0 mL) + 1 vial CSE (500 ng/vial)		
Catalog number	Gel Clot Sensitivity EU/mL	Kinetic Turbidimetric Assay Quantitative Range (EU/mL)
WPEK4-20015	0.015	0.001 to 10
WPEK4-20003	0.03	
WPEK4-20006	0.06	
WPEK4-20125	0.125	
WPEK4-20025	0.25	

PYROSTAR™ ES-F 5.2 mL Test Kit • 4 multi-test vials (5.2 mL) + 1 vial CSE (500 ng/vial)		
Catalog number	Gel Clot Sensitivity EU/mL	Kinetic Turbidimetric Assay Quantitative Range (EU/mL)
WPEK4-50015	0.015	0.001 to 10
WPEK4-50003	0.03	
WPEK4-50006	0.06	
WPEK4-50125	0.125	
WPEK4-50025	0.25	

Single- Test Lysate with matched CSE

PYROSTAR™ ES-F Single Test Kit • 25 single-test vials + 1 vial CSE (500 ng/vial)		
Catalog number	Gel Clot Sensitivity EU/mL	Kinetic Turbidimetric Assay Quantitative Range (EU/mL)
WPESK-0015	0.015	0.001 to 10

Bulk Lysate

PYROSTAR™ ES-F 2.0 ml Bulk Kit • 100 multi-test vials (2.0 mL)		
Catalog number	Gel Clot Sensitivity EU/mL	Kinetic Turbidimetric Assay Quantitative Range (EU/mL)
WPEM-20015	0.015	0.001 to 10
WPEM-20003	0.03	
WPEM-20006	0.06	
WPEM-20125	0.125	
WPEM-20025	0.25	

Bulk Lysate

PYROSTAR™ ES-F 5.2 ml Bulk Kit • 100 multi-test vials (5.2 mL)		
Catalog number	Gel Clot Sensitivity EU/mL	Kinetic Turbidimetric Assay Quantitative Range (EU/mL)
WPEM-50015	0.015	0.001 to 10
WPEM-50003	0.03	
WPEM-50006	0.06	
WPEM-50125	0.125	
WPEM-50025	0.25	

PYROSTAR™ ES-F/Plate 2 mL Test Kit • 4 multi-test vials (2 mL) + 1 vial CSE (500 ng/vial)		
Catalog number	Gel Clot Sensitivity EU/mL	Kinetic Turbidimetric Assay Quantitative Range (EU/mL)
WPEPK4-20015	0.015	0.01 to 10

Our Promise

As an FDA licensed facility, Wako Chemicals USA, Inc. – LAL Division is committed to ensuring that our production site and LAL reagents comply with all the rules, regulations, and quality standards set forth by FDA for current Good Manufacturing Practices (cGMP's).

Horseshoe Crab Conservation



Wako Chemicals USA, Inc. is very much concerned with maintaining the viability of the horseshoe crab population. We are dedicated to following practices that ensure the careful handling and good quality of crabs used for LAL manufacture that both minimize injury and protect this invaluable species. After bleeding, the crabs are returned the next day by our fishermen to the same waters where they were collected. To assist in the collection of data for crab conservation studies, Wako USA participates in a horseshoe crab tagging and monitoring program coordinated by the U.S. Fish and Wildlife Service.



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