FUJIFILM
Value from Innovation

Wako

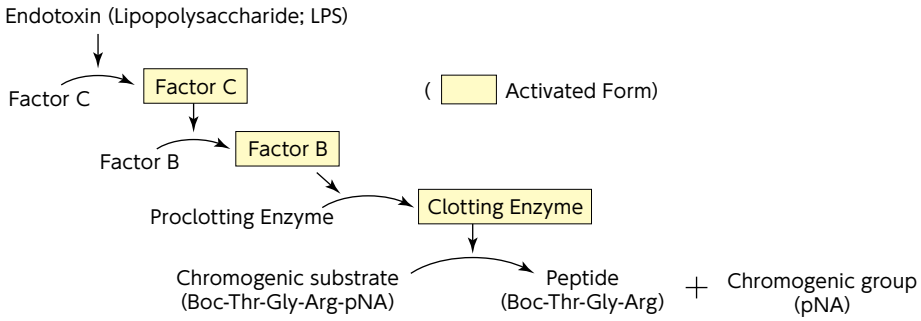
Recombinant Cascade Reagent (rCR) for Bacterial Endotoxin Test

PYROSTAR™ Neo+

Endotoxin, a major pyrogen, is commonly known as lipopolysaccharide (LPS) that is a component of the cell wall of Gram-negative bacteria. If parenteral drugs are contaminated with endotoxin, it may cause serious symptoms such as fever and shock. Bacterial Endotoxin Testing (BET) is performed using a Limulus Amebocyte Lysate (LAL) reagent made from horseshoe crab blood extracts. This is based on the phenomenon that horseshoe crab amebocyte coagulates in the presence of endotoxin. PYROSTAR™ Neo+ a horseshoe crab-free reagent for BET consists of recombinant Factor C, Factor B, and proclotting enzyme which are the main components of LAL reagent, as well as chromogenic synthetic substrate, and buffer components. Endotoxin can be measured by the chromogenic substrate method in the same way as the conventional LAL reagent.

Principle

PYROSTAR™ Neo+ contains three recombinant proteins, horseshoe crab Factor C, Factor B, and proclotting enzyme, and chromogenic synthetic substrate t-Boc-Thr-Gly-Arg-pNA. When endotoxin is present in the sample to be tested, endotoxin activates Factor C and subsequent cascade reaction occurs as shown in the figure below, resulting in cleavage of the chromogenic substrate and release of para-nitroaniline (pNA). The amount of endotoxin in the sample is determined by the relationship between the standard endotoxin concentration and time which takes for the absorbance to reach a predetermined value, i.e., the time which takes for the amount of free pNA to reach a certain concentration.



Features

- Less variations in reactivity between reagent lots by utilizing recombinant proteins instead of horseshoe crab amebocyte.
- Equivalent reactivity and repeatability to conventional LAL reagents.
- Endotoxin-specific [No Factor G is included which is activated by (1→3)- β -D-glucan and activate proclotting enzyme.]
- Colorimetric quantification with a microplate reader or Toxinometer®.

How to use

Reagent preparation

PYROSTAR™ Neo+

Lyophilized formulation

2.7 mL
Water for BET

Measurement

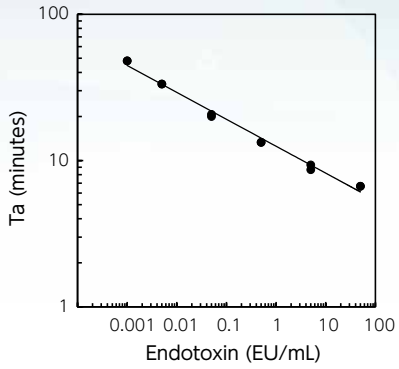
Sample : 50 μL + Reagent : 50 μL
Add to microplate

Microplate reader

Sample : 100 μL + Reagent : 100 μL
Add to test tube

Toxinometer®

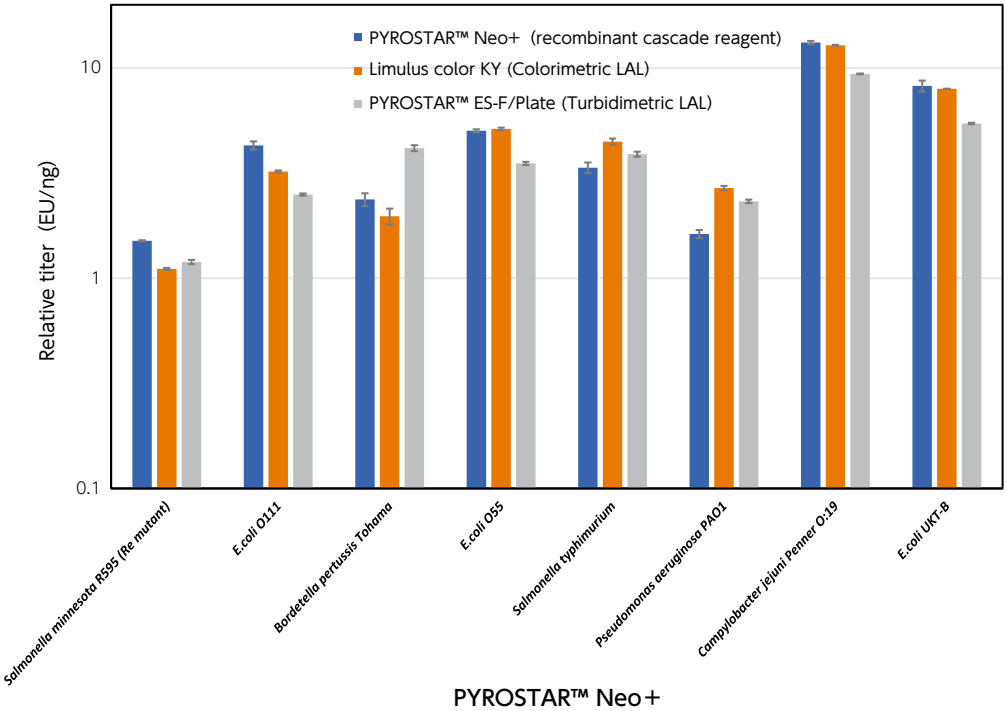
Standard curve



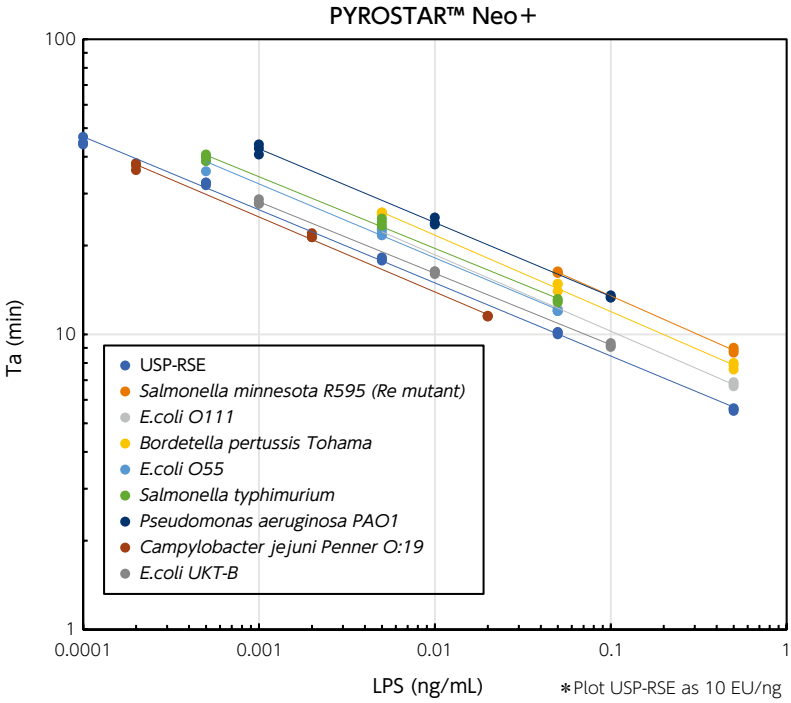
Create standard curve using the activation times obtained from the standard endotoxin. Calculate the endotoxin concentration of the sample from the activation times of the sample and the standard curve. X-axis is the endotoxin concentration on logarithm and Y-axis is the activation time on logarithm. Linear or quadratic regression can be used.

High sensitivity
Quantitative range: 0.001 - 50 EU/mL

Reactivity to LPS from different bacterial species



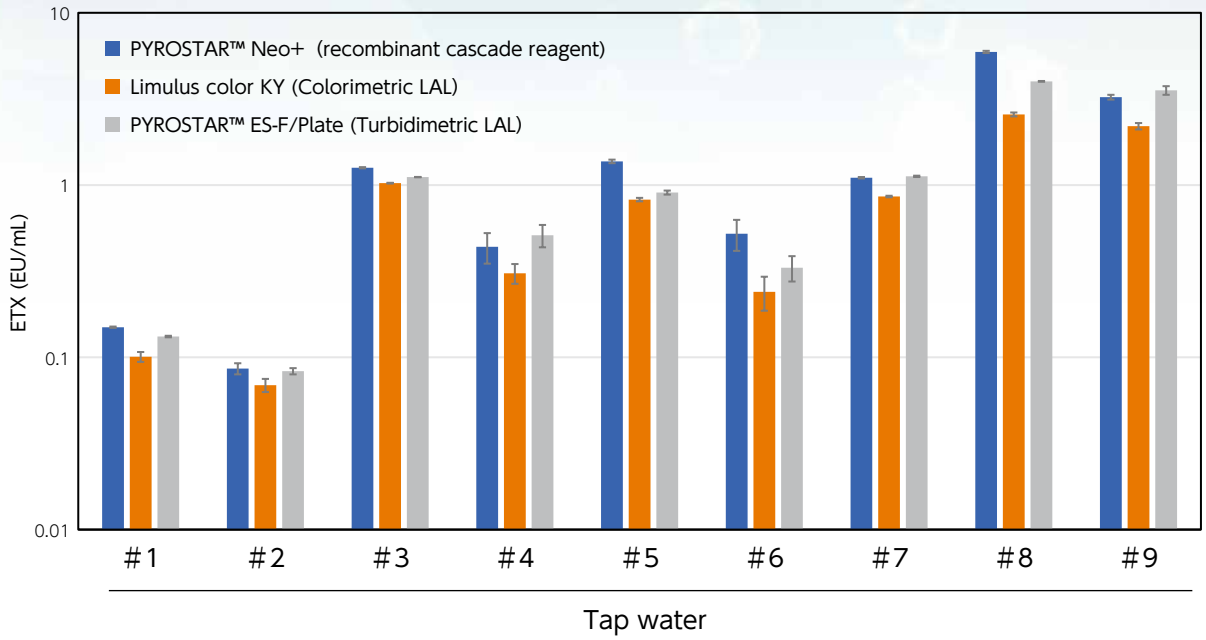
The relative titer of LPS from various bacterial species against USP-RSE measured with PYROSTAR™ Neo+ was equivalent to the titer measured with LAL reagents.



It also showed good parallelism with USP-RSE.

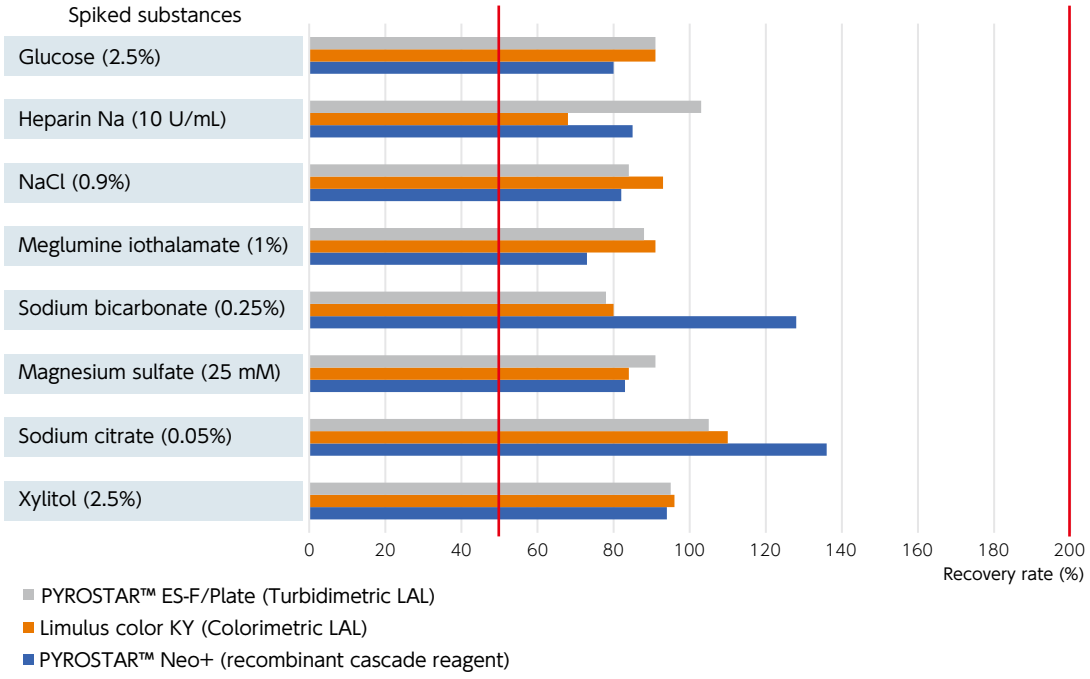
PYROSTAR™ Neo+ can measure not only standard endotoxin but also LPS from various bacterial species with good equivalency to LAL reagents.

Reactivity to Naturally Occurring Endotoxin (NOE)



PYROSTAR™ Neo+ has equivalent reactivity against NOE compared to LAL.

Spike recovery test with drug substances



PYROSTAR™ Neo+ showed good recovery rate as well as LAL.

Product Information

Product code	Product name	Size	Storage
293-36941	PYROSTAR™ Neo+	50 tests *	2-10℃

* For microplate reader : 50 tests
For Toxinometer : 25 tests

Related product

Tips for endotoxin test

Product code	Product name	Size
294-35011	Bio-Clean Tip WAKO® EXTEND S Ⅱ	100 tips
291-35021	Bio-Clean Tip WAKO® 200 Ⅱ	100 tips
298-35031	Bio-Clean Tip WAKO® 1000 Ⅱ	100 tips

Plate for endotoxin test

Product code	Product name	Size
Corning #3595	96-well Clear Flat Bottom Polystyrene TC-treated Microplate, Individually Wrapped, with Low Evaporation Lid, Sterile	50 plates

Test tubes and aluminum caps for endotoxin testing

Product code	Product name	Size
292-32751	Limulus Test Tube-S with Aluminum Cap	10 pcs × 8
293-26551	Limulus Test Tube-S	10 pcs × 10
293-28251	Aluminum Cap-S	10 pcs × 10

Instrumentation for endotoxin measurement

Product code	Product name	Size
293-36061	Toxinometer® ET-7000	1 unit
—	EPOCH2 Absorbance Plate Reader	1 unit
290-36831	Toximaster™ FQC1 Software PC Set E	1 set

For Non-endotoxin Pyrogens detection

Monocyte Activation Test (MAT) : *In vitro* alternative method of Rabbit Pyrogen Test.

Product code	Product name	Size
298-36991	LumiMAT™ Pyrogen Detection Kit - Cells	96 tests
297-96801	LumiMAT™ Pyrogen Detection Kit - Reagent Set	96 tests
292-37011	Toximaster™ FQC1 Software PC Set E for LumiMAT™	1 set

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