

# LumiMAT™ Pyrogen Detection Kit

LumiMAT™ is a Monocyte Activation Test (MAT) to detect pyrogens. Pyrogen is a generic term for substances that cause body temperature rise in animals and humans. Parenteral drugs and medical devices which get in contact with patient's blood system must be free from pyrogens.

The first developed test to detect a wide range of pyrogens was Rabbit Pyrogen Test (RPT). In RPT, samples to be tested are intravenously administered to rabbits and monitor the body temperature. Since RPT has problems with reproducibility, accuracy, and cost, Limulus Amebocyte Lysate (LAL) reagent has been widely used as the replacement. However, LAL reagent cannot detect non-endotoxin pyrogens, and RPT is still used in case LAL reagent is not appropriate.

MAT is an in vitro pyrogen test developed as an alternative of RPT and it can detect not only endotoxin but also non-endotoxin pyrogens.

		RPT	LAL	MAT
Pyrogen	Endotoxin (derived from Gram-negative bacterial cell walls)	✓	✓	✓
	Non-endotoxin Pyrogens (derived from Gram-positive bacteria, viruses, fungi etc.)	✓	Not-detectable	✓

## Assay principle

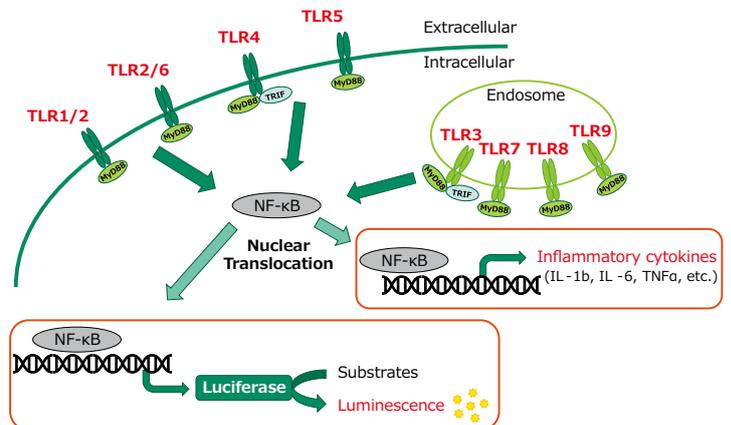
MAT is used to detect pyrogens that activate human monocytic cells to release inflammatory cytokines.

### ■ Conventional MAT

Culture PBMCs (peripheral blood mononuclear cells) and samples to be tested in microwell plate. Cytokines released from monocytic cells by exposure to pyrogens are measured by ELISA to detect pyrogens in the samples.

### ■ LumiMAT™

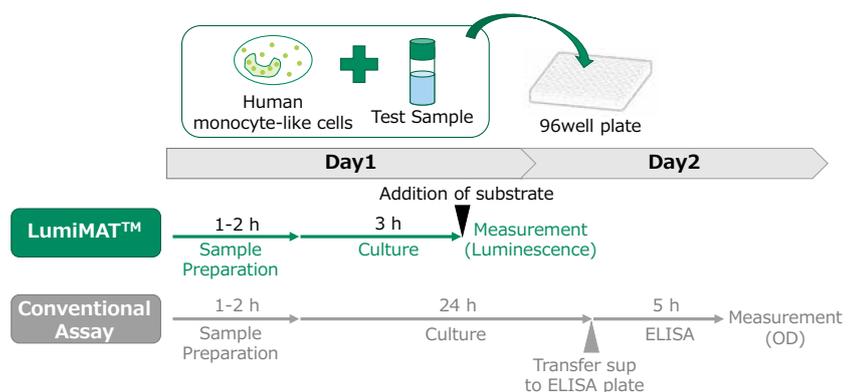
LumiMAT™ uses a monocytic cell line NOMO-1 in which a luciferase reporter gene is introduced to express luciferase protein in response to NF-κB signals activated by exposure to pyrogens. Because the expressed luciferase generates luminescence by reacting with the substrate, luminescence can be detected by a luminescence microplate reader to detect pyrogens in the samples.



## Assay Flow

Add samples to be tested or standard endotoxin into microplate and add reporter cells and incubate for 3 hours. After the incubation add luminescent substrate to measure luminescence with a plate reader.

Compared to ELISA, this is a simple oneplate assay that suppresses inter-test variations. In addition, results can be obtained in about 5 hours, which is significantly shorter than the 1.5 days required by the conventional method.



# Features of LumiMAT™

## Conventional method

### Assay with human blood-derived PBMCs

Testing time: 1.5 days

-Over night incubation

Detection: ELISA

-Measurement of released cytokines by ELISA

-Time and labor consuming

Cells: PBMC

-Lot-to-lot differences due to different donors

-Concerns about stable supply

## LumiMAT™

### Assay with reporter NOMO-1 cells

Rapid testing (5 hours)

-3 hours incubation

Convenient reporter assay

-Only add luminescent substrate to the plate after incubation

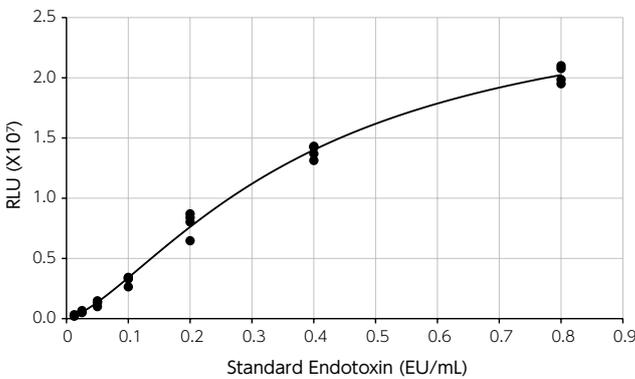
Stable reporter cell line

-Cell lines stably expressing reporter gene

-High reproducibility with less lot-to-lot variation

-Stable supply from master cell bank

## Standard curve



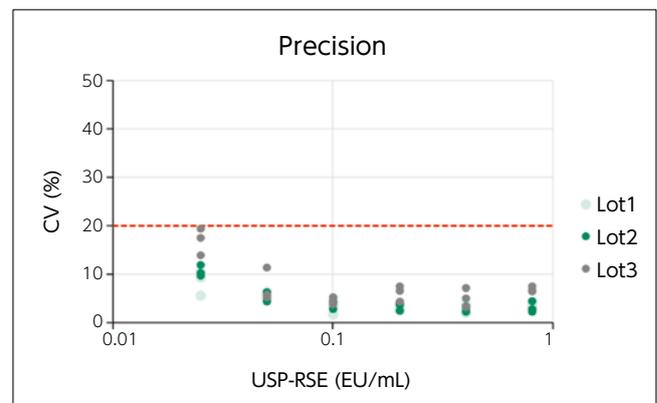
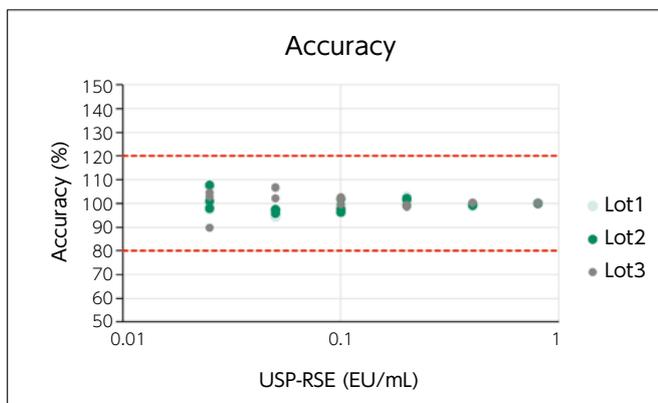
Create standard curve using reference standard endotoxin and calculate the concentration of pyrogens as Endotoxin Equivalents (EE)/mL.

Standard curve range: 0.0125 ~ 0.8 EE/mL

RLU : Relative Light Unit  
EU : Endotoxin Unit

## Reproducibility

Accuracy and precision were measured with three different lots of the LumiMAT cells (n=4, 3 assays for each lot). Six concentrations of standard endotoxin (USP-RSE) were spiked. Measured the concentrations of endotoxin (EU/mL) of the spiked samples with the standard curve and calculated accuracy and precision.



All lots had high reproducibility.

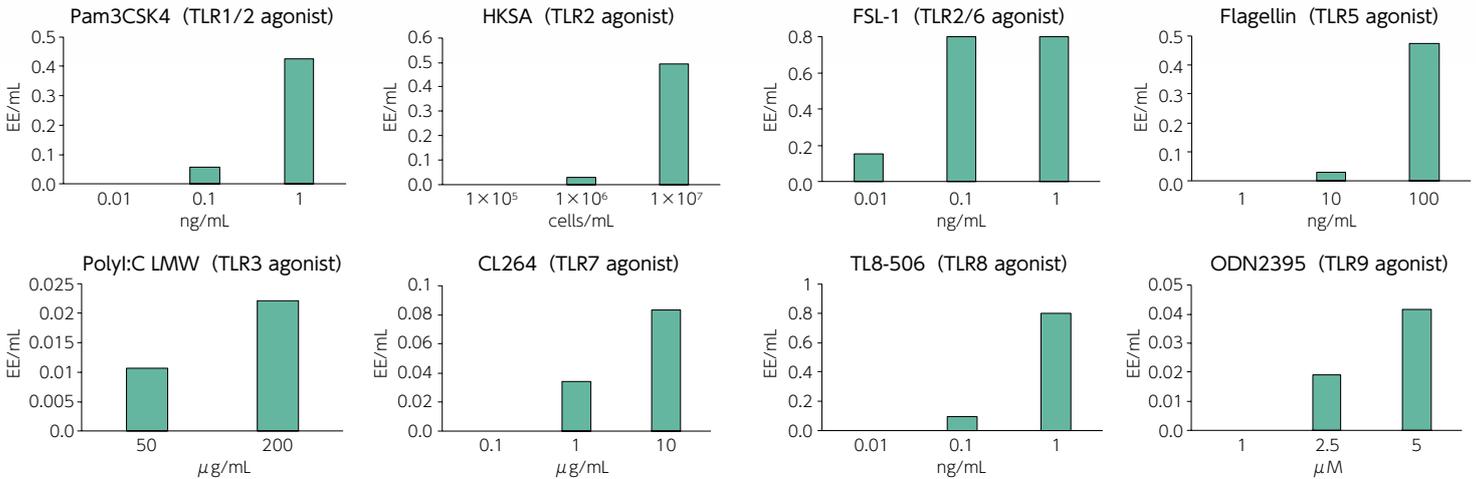
Accuracy (measured value/true value) : within  $\pm 10\%$

Precision (CV of measured value) : within 20%

## Reactivity to Non-Endotoxin Pyrogens (NEPs)

NEPs which react with each Toll-like receptor (TLR) were added at several concentrations, and the concentration of each NEP was calculated as EE/mL from the standard curve.

Y axis: EE/mL, X axis: Spiked concentration of NEP



All tested NEPs were detected.

## Spike recovery test

### 1. Spike recovery test using pharmaceutical products

Standard endotoxin was added to each drug product and the recovery rate was calculated.

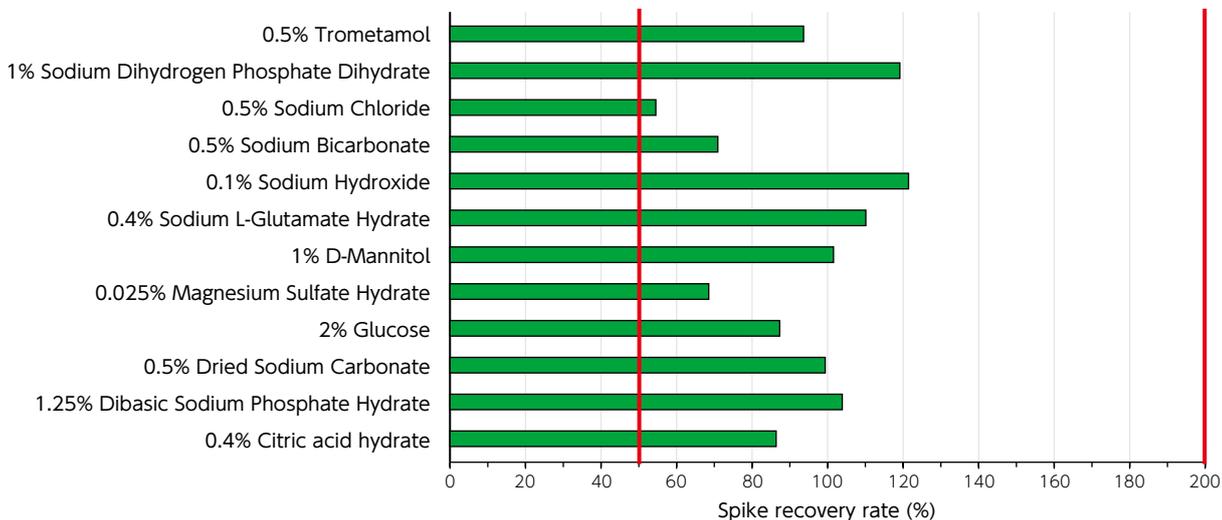
Drug	MVD *	Fold-dilution	Spike recovery (%) n=3			Interference **
			0.1 EU/mL	0.4 EU/mL	0.8 EU/mL	
Albumin 25% I.V. 5 g/20 mL	480	10	121.6	98.9	97.5	N
		100	134.8	119.4	110.3	N
		400	109.5	110.3	108.0	N
Aciclovir 25 mg/mL	500	10	67.5	63.7	50.2	N
		100	89.7	87.8	82.3	N
		400	96.0	95.6	99.2	N
Epoetin Alfa 750 I.U./0.5 mL	12000	100	99.3	103.1	105.2	N
		500	92.9	100.5	102.6	N
		1000	87.9	98.0	98.9	N
Romozumab 105 mg/1.17 mL	10200	100	109.9	110.5	112.6	N
		500	94.9	94.3	98.6	N
		1000	95.0	92.0	92.3	N

\* MVD : Maximum Valid Dilution    \*\* No interference :  $50 < \text{Recovery} (\%) < 200$



Recovery rate were within 50-200% for all four pharmaceuticals.

### 2. Spike recovery test using pharmaceutical raw materials



LumiMAT™ is applicable for a variety of samples.

## Product Specification

Cell concentration	$5 \pm 2 \times 10^6$ cells/vial
Cell viability	$\geq 85$ % of the time
LPS LOD	$\leq 0.0125$ EU/mL
LPS standard curve $R^2$	$\geq 0.975$
Pam3CSK4 10 ng/mL	$\geq 0.05$ EE/mL

HKSA $1 \times 10^8$ cells/mL	$\geq 0.05$ EE/mL
Flagellin $1 \mu\text{g/mL}$	$\geq 0.05$ EE/mL
Mycoplasma test	Negative
Endotoxin test	$< 0.0125$ EU/mL

## Kit Components

LumiMAT™ Pyrogen Detection Kit - Reagent Set	
Assay medium	20 mL
Dilution medium	100 mL
Luciferase assay buffer	12 mL
Luciferase substrate	240 $\mu\text{L}$

LumiMAT™ Pyrogen Detection Kit - Cells	
LumiMAT™ Cells	350 $\mu\text{L}$

\* Both LumiMAT™ Pyrogen Detection Kit - Cells and Reagent Set are necessary for an assay.

## Status of MAT in Pharmacopoeias

Pharmacopeia	Status
18 <sup>th</sup> Japanese Pharmacopoeia	- Under validation by JaCVAM (Japanese Center for the Validation of Alternatives Methods)
European Pharmacopoeia (Ph. Eur.)	+ General Chapter 2.6.30 (since 2010) RPT will be removed by 2026.
United States Pharmacopoeia (USP)	- Alternative method on FDA guidance
Chinese Pharmacopoeia (ChP)	+ since ChP2020
Indian Pharmacopoeia (IP)	+ since IP2018

## Product Information

Product code	Product name	Size	Storage
297-96801	LumiMAT™ Pyrogen Detection Kit - Reagent Set	96 Tests	-20°C
298-36991	LumiMAT™ Pyrogen Detection Kit - Cells	96 Tests	-80°C

## Related Product

Product code	Product name	Size
292-37011	Toximaster™ FQC1 Software PC Set E for LumiMAT™	1 set

Toximaster™ FQC1 Software for LumiMAT™ is applicable to TECAN Infinite® 200 PRO Lumi and Promega GloMax® Navigator/Explorer/Discover System.



### Assay procedure video

Access via QR code or search for product code No. 297-96801 on Fujifilm Wako Laboratory Chemicals site.

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