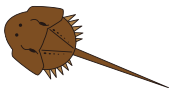
FUJIFILM
Value from Innovation

Wako

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

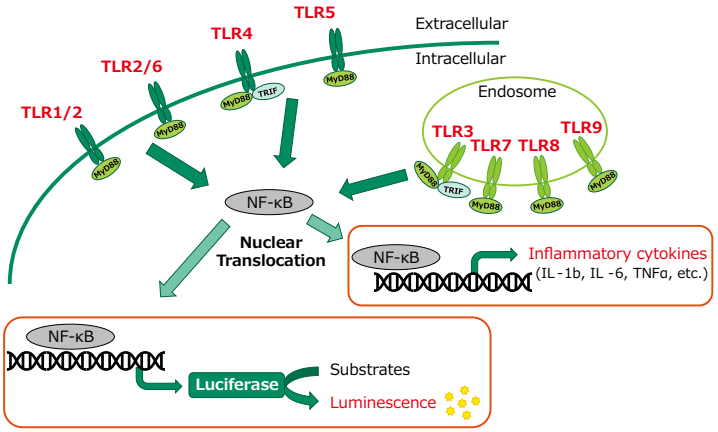
MAT is used to detect pyrogens that activate human monocytic cells to release inflammatory cytokines.

■ 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.

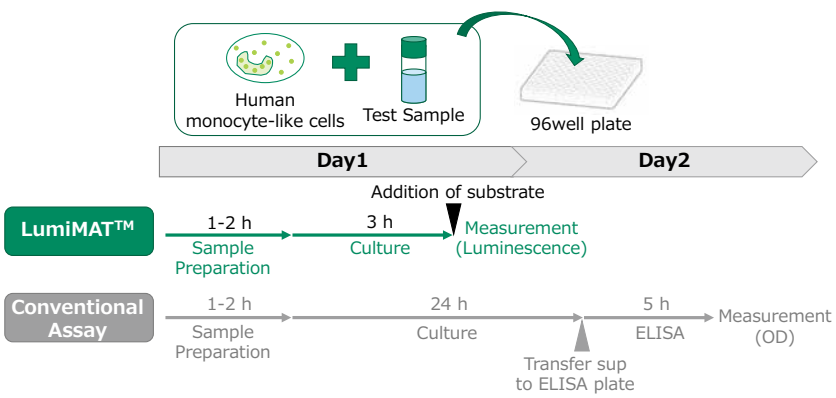
■ LumiMAT™

LumiMAT™ uses a monocytic cell line NOMO-1 in which a luciferase reporter gene is introduced to express luciferase protein in response to NF-kB 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

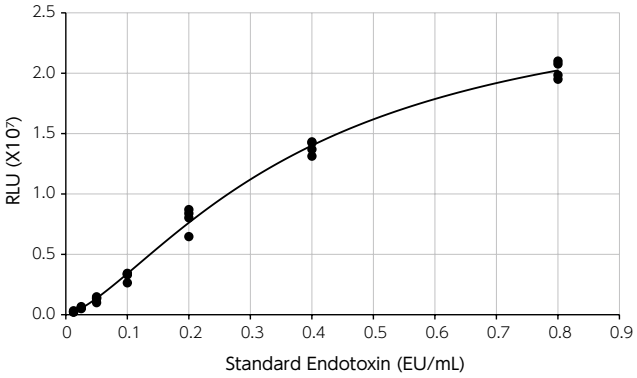
Add samples to be tested or standard endotoxin into microplate and add reporter cells and incubate for 3 hours. After the incubation add luminescent substrate to measure luminescence with a plate reader.
Compared to ELISA, this is a simple oneplate assay that suppresses inter-test variations. In addition, 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	LumiMAT™
Assay with human blood-derived PBMCs	Assay with reporter NOMO-1 cells
Testing time: 1.5 days -Over night incubation	Rapid testing (5 hours) -3 hours incubation
Detection: ELISA -Measurement of released cytokines by ELISA -Time and labor consuming	Convenient reporter assay -Only add luminescent substrate to the plate after incubation
Cells: PBMC -Lot-to-lot differences due to different donors -Concerns about stable supply	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



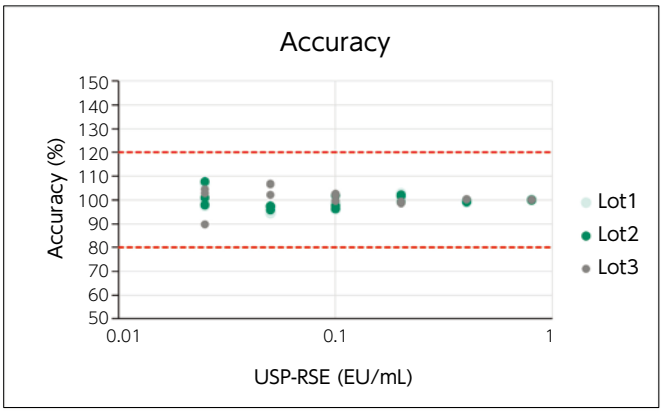
Create standard curve using reference standard endotoxin and calculate the concentration of pyrogens as Endotoxin Equivalents (EE)/mL.

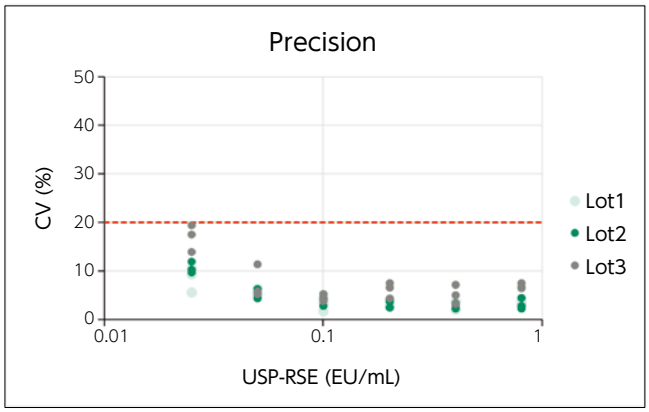
Standard curve range: 0.0125 ~ 0.8 EE/mL

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.

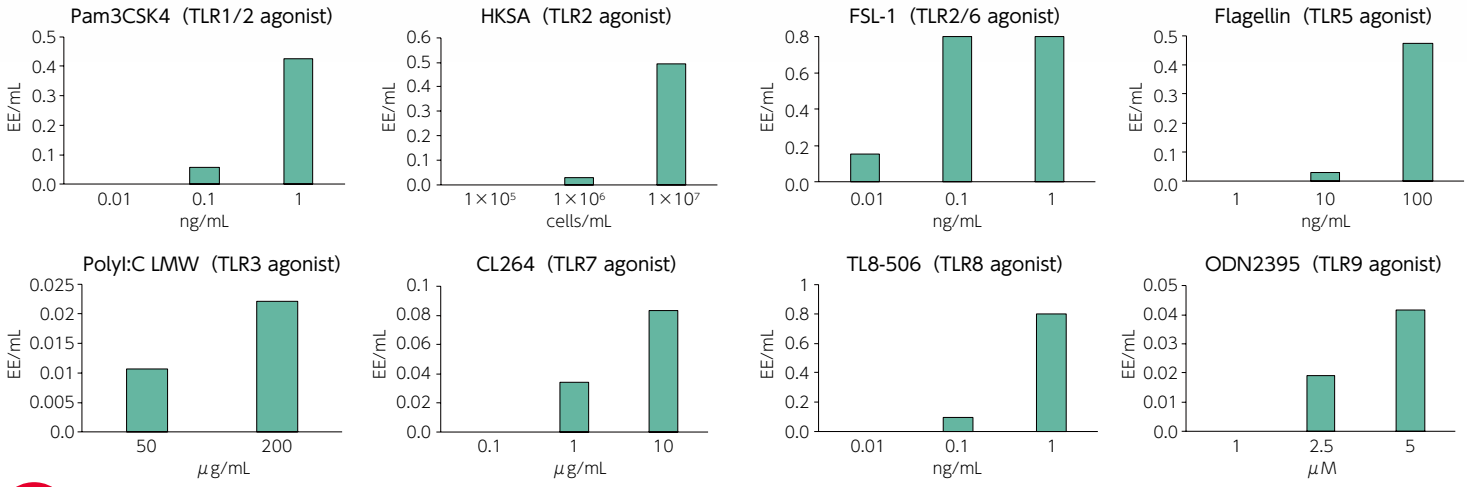




All lots had high reproducibility.
Accuracy (measured value/true value) : within ± 10%
Precision (CV of measured value) : within 20%

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

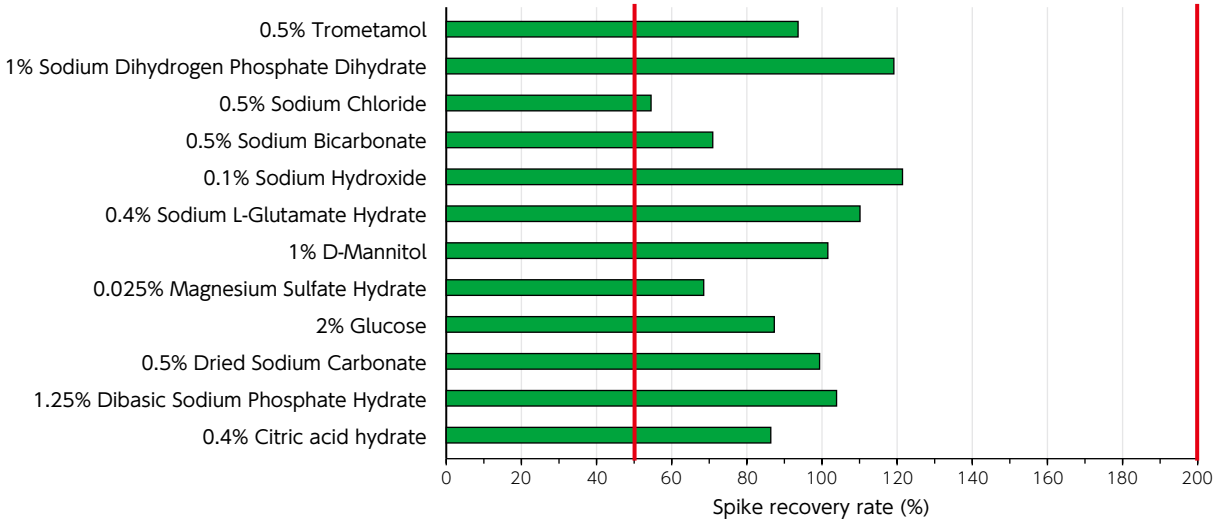
Standard endotoxin was added to each drug product and the recovery rate was calculated.

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
Romosozumab 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 < 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 ± 2 × 10^6 cells/vial
Cell viability	≧ 85 % of the time
LPS LOD	≦ 0.0125 EU/mL
LPS standard curve R^2	≧ 0.975
Pam3CSK4 10 ng/mL	≧ 0.05 EE/mL

HKSA 1 × 10^8 cells/mL	≧ 0.05 EE/mL
Flagellin 1 μg/mL	≧ 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 μL

LumiMAT™ Pyrogen Detection Kit - Cells	
LumiMAT™ Cells	350 μL

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

Status of MAT in Pharmacopoeias

Pharmacopeia	Status	
18th 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 Pharmacopeia (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℃
298-36991	LumiMAT™ Pyrogen Detection Kit - Cells	96 Tests	−80℃

Related Product

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

Assay procedure video

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

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 Europe GmbH
Fuggerstr 12, 41468 Neuss, Germany
Tel: +49 2131 311 0 Fax: +49 2131 311 100
labchem_wkeu@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 (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

24700L$_{01}$K