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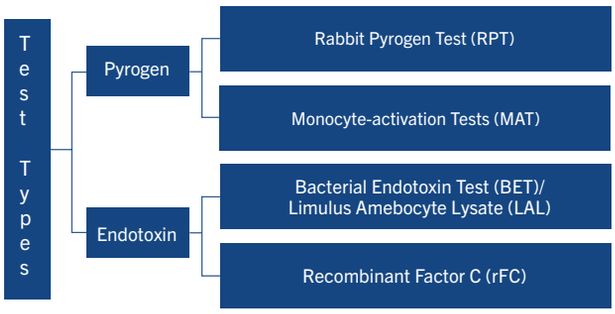
MONOCYTE ACTIVATION TEST (MAT) – Not all MAT tests are the same

Pyrogen testing becomes animal-free

Pyrogens are heat-stable substances derived from bacteria, fungi, or viruses that can induce fever in humans. As such contaminations with pyrogens in parental pharmaceuticals pose life-threatening risks, thus the degree of contamination is a critical quality control measure to ensure patient safety.

Pyrogens are classified as endotoxins and non-endotoxin pyrogens (NEPs). In the 20th century the rabbit pyrogen test (RPT) and bacterial endotoxin test (BET) represented the standard pyrogen testing methods. In recent years, the monocyte activation assay (MAT) has been developed as an in vitro alternative for RPT, simulating the human immune response to pyrogens.

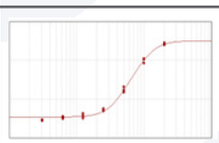
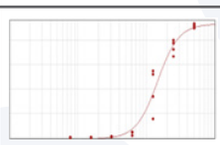
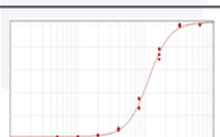
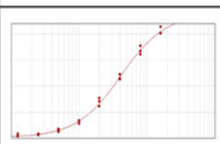
In 2010 the MAT was introduced as the alternative compendial method for pyrogen testing in the European Pharmacopoeia (EP, chapter 2.6.30). In 2021 the EP commission even decided to aim for a complete replacement of the RPT by July 2025 with a clear preference for the MAT.



Eurofins offers state-of-the-art animal-free solutions and helps you with the transition

As the Rabbit Pyrogen Test (RPT) is being phased out for ethical reasons, Eurofins BioPharma Product Testing Germany has implemented several commercially available Monocyte Activation Test (MAT) kits in accordance with current EP guidelines to meet diverse customer requirements.

These MAT kits from various suppliers use different cells and readouts, though the basic principle remains consistent: the test sample is incubated with cells (cell line or peripheral blood mononuclear cells, PBMC) to activate the immune response (e.g. IL-6 or TNF-alpha), which is then quantified with e.g. ELISA. Below is an overview of the established MAT kits, with key parameters and performance results:

MAT Kit	1	2	3	4
Cells	PBMC	PBMC	THP-1 macrophage cell line	NOMO-1 reporter cell line
Readout	IL-6	IL-6	TNF-alpha	NF-kB
Test Duration	2 days	2 days	3 days	1 day
Detection of NEP	✓	✓	✓	✓
LOD	0.25 EU/mL	0.02 EU/mL	0.02 EU/mL	0.03 EU/mL
Recovery Range	99-110%	58-118%	73-101%	83-105%
Intermed. Precision*	Not determined	2-32%	11%	7%
Repeatability*	Not determined	2-5%	2-25%	7-16%
Exemplary standard curve				

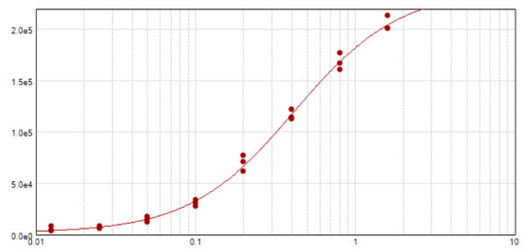
* These parameters were tested for the LPS control and selected NEPs. LOD = Limit of Detection

MAT kits are evolving and accelerating - Novel MAT approach

We were privileged to use a next-generation MAT kit (#4 in the overview) before market launch. By using a reporter gene cell line, the assay is completed within one working day and consists of five steps:

- Dilution of the reference standard (LPS), controls (LPS + NEP) and sample
- Thawing and addition of the cells
- Incubation for 5h at 37°C
- Addition of the detection solution
- Measurement of Luminescence

The concentration (Endotoxin units per mL, EU/mL) for the controls and samples are calculated using a 4PL fitted standard curve.



y-axis: Luminescence, x-axis: Concentration in EU/mL, R2=0.996

Intermediate precision was calculated for the LPS control between three experiments on different days:

Accuracy of the LPS control [%Recovery]				Intermediate Precision [%CV]
Experiment 1	Experiment 2	Experiment 3	Mean	
106	90	103	100	7

To determine repeatability three sets of LPS control and three NEPs were analysed in one experiment:

	Determined EU/mL				Repeatability [%CV]
	Set 1	Set 2	Set 3	Mean	
BRP (0.5 EU/mL)	0.52	0.41	0.52	0.48	10
Flagellin	0.03	0.03	0.03	0.03	10
Lipoteichoic aid	0.18	0.23	0.16	0.19	16
Peptidoglycan	0.25	0.30	0.26	0.27	7

Our results have shown that this next-generation MAT kit provides results much faster, with comparable quality to other commercially available MAT kits.

Ethical and reliable pyrogen testing in Germany
Switch to the Monocyte Activation Test today for a safer, animal-free approach to pyrogen testing. MAT offers the benefits of both the RPT and the BET, including:

- Quantitative, sensitive, and reproducible results
- Detection of both endotoxins (LPS) and non-endotoxin pyrogens (NEPs)
- Ethical, animal-free testing environment
- Accepted by regulatory bodies (FDA, EMA) as a valid alternative test

We are here to share our expertise and help you select the MAT solution best suited to your product needs. Contact us with any questions – we're happy to assist!

Contact Us:

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