NAT-MAT®

Next Generation Pyrogen Testing

Combining the Power of Digital PCR with MAT for Enhanced Precision and Sensitivity

- ✓ Ph. Eur. 2.6.30 compliant
- ✓ Replacing the Rabbit Pyrogen Test
- ✓ Excellent reproducibility
- ✓ Highly automated workflow
- ✓ Compatible with QIAcuity platform
- ✓ Time-to-result: 1 day
- ✓ Analysis of results with easy-to-use software

NAT-MAT® Pyrogen Testing

Get a head start on Ph. Eur. mandated RPT transition

In the pharmaceutical industry, pyrogen detection is mandatory to avoid life-threatening fever reactions that can be induced by both microbial and non-microbial substances. Traditionally, this test relied on animal-based methods, like the rabbit pyrogen test (RPT). With increasing awareness of ethical concerns and growing demand for animal-free alternatives, the Monocyte Activation Test (MAT) was introduced into the *European Pharmacopeia (EP)* in 2010 – providing a human in vitro system to detect and quantify pyrogenic substances in pharmaceutical products. Since the RPT will be discontinued in Europe in 2026, regulatorry authorities are currently encouraging manufacturers to integrate MAT into their QC system.

What is the NAT-MAT® and how does it work?

The MAT is based on the activation of monocytes by pyrogenic substances present in the sample. Upon exposure to potential pyrogens, monocytes undergo a signalling pathway resulting in secretion of pro-inflammatory cytokines, such as IL-1β, TNF-α and IL-6.
Minerva Biolabs has developed a next generation MAT system that measures the gene expression of IL-1β and TNF-α using digital PCR – the NAT-MAT®. The protocol is optimized for fast and reliable pyrogen detection and offers robust and highly sensitive results. The NAT-MAT® enables complete pyrogen testing and detects endotoxin as well as non-endotoxin pyrogens. The test can be conducted as In-Process control and as final release testing of medicinal products according to *Ph. Eur.* 2.6.30.
Due to the parallel measurement of two cytokines and one housekeeping gene more accurate results can be generated. The housekeeping gene also works as quality control of extraction and cell number, thus the assay functions within a range of cell densities.
Analysis of the results can be done automatically by Minerva Biolabs' NAT-MAT® software according to the requirements of *Ph. Eur.* 2.6.30.

Simple and fast workflow

1. Seeding & stimulation

Seed **NAT-MAT® Cells**, incubate and stimulate with test sample for 4 hours

2. Extraction

Extract nucleic acids with the beads-based **NAT-MAT® SP**

3. Detection

Detect cytokines by dPCR with **NAT-MAT® dPCR**

4. Analysis

Calculate pyrogen load in your test sample with [IU/ml] **NAT-MAT® Analysis Software**

Issues with existing ELISA MAT	Benefits with Minerva Biolabs' NAT-MAT®
Reproducibility issues due to high variance in PBMCs	Reproducible results by utilizing a monocytic cell line
No normalisation of pyrogenic load due to undefined ratio between cell number and detected pyrogens	Normalisation of pyrogenic load by parallelly measuring the gene expression of a housekeeping gene – more accurate results
Cell supply ability	Using a cell line ensures good cell supply ability
Management of donor information in terms of ethics	No ethical issues regarding donor information since a cell line is used instead of PBMCs
Test takes a long time (2 days)	Fast workflow (1 day)

What do I need to perform pyrogen testing?

The NAT-MAT® system offers you all necessary components for conducting complete pyrogen testing:

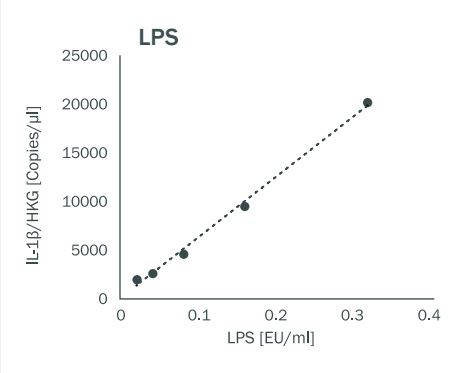
1. NAT-MAT® Cells
NAT-MAT® Cells is a vial of HL-60 derived macrophages that are ready for seeding into a 96-well plate. These cells were produced by differentiation of HL-60 myeloblasts with phorbol-12-myristate-13-acetate (PMA).

2. NAT-MAT® SP
NAT-MAT® SP is a magnetic beads extraction kit and has been developed for the automated extraction of nucleic acids from NAT-MAT® Cells in 96-well format (extraction can also be done manually).

3. NAT-MAT® dPCR
NAT-MAT® dPCR amplifies two cytokines (IL-1β, TNF-α) and one housekeeping gene. The kit includes all reagents required for the dPCR analysis. Primer, probes, nucleotides and polymerase are provided in a ready-to-use lyophilized reaction mix. Pipetting of the PCR reactions into the nanoplate can be done automated by using a pipetting robot or manually.

Detecting Endotoxin and Non-Endotoxin Pyrogens

Complete pyrogen testing require the detection of endotoxin and non-endotoxin pyrogens. Certain pyrogen standards have been tested with the NAT-MAT® system, showing excellent linearity.



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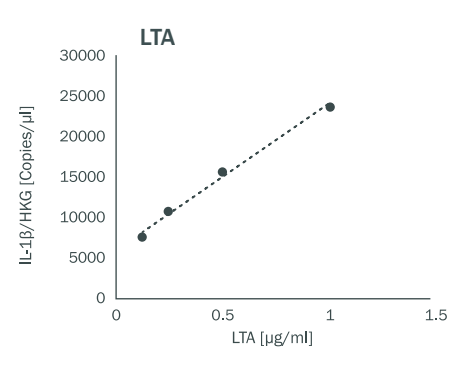
Correlation [R2]: 0.9957



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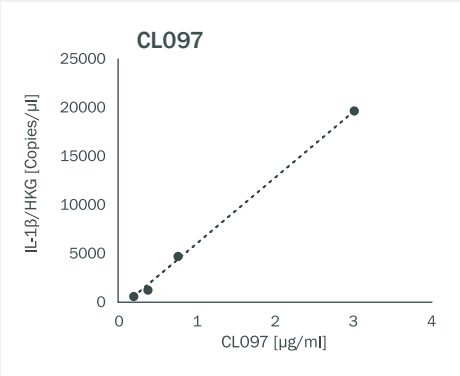

Slope factor: 6847
Correlation [R2]: 0.9984

Figure 1: Standard curve of Lipopolysaccharide (LPS) as endotoxin pyrogen, Lipoteichoic acid (LTA) and CL097 as non-endotoxin pyrogen (y-axis: IL-1β/housekeeping gene ratio [copies/µl]; x-axis: different concentrations of pyrogen standard [µg/ml])

Establishing the NAT-MAT® in the lab is relatively straightforward. European manufacturers simply need to assess the feasibility of NAT-MAT® for their product and then proceed with a product specific validation. It is possible that laboratories will complete these studies within about 6 to 8 weeks.

For more information about Minerva Biolabs' NAT-MAT® solution get in touch with our pyrogen testing experts.

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