MERCK

The human(e) alternative in pyrogen testing

PyroDetect System

Millipore®

Preparation, Separation, Filtration & Testing Products

Principle of the PyroDetect System

Broad pyrogen profile, easy assay procedure and robust application

Merck is the leading provider of microbiology products in the fields of hygiene monitoring and product testing. Being aware of the extreme importance of efficient pyrogen testing within the pharmaceutical industry, we have further broadened its product portfolio with the new PyroDetect System, a patented assay procedure.

Pyrogens in pharmaceutical products, medical devices, biotherapeutics and cosmetics can induce life threatening fever. Thus it is mandatory for the respective industry to ensure that the pyrogen concentrations do not exceed the contaminant limits specific to each product.

The Rabbit Pyrogen Test and the Limulus Amebocyte Lysate (LAL) test are broadly used for the detection of pyrogens. Both methods are limited in the products which can be analysed and both have a high level of animal consumption. In the case of the LAL test, only endotoxins can be detected causing a safety risk.

To overcome these limitations, the Monocyte-Activation Test (MAT) was introduced in the European Pharmacopoeia in 2010 as an alternative to the Rabbit Pyrogen Test (EP Chapter 2.6.30). Using whole human blood, the PyroDetect System mimics the innate immune response of a fever reaction caused by pyrogens. The MAT test simulates this response better than any animal test.

Comparison of Pyrogen Test Methods

Test principle	Fever reaction in mammal	Defence mechanism	Fever reaction in human
	Rabbit	LAL	PyroDetect
Contamination			
Gram-negative	+	+	+
Gram-positive	+	-	+
Yeasts & Molds	+	-	+
Virus	+/-[1]	-	+
Application			
Pharmaceuticals	+	+	+
Biologicals (e.g. gene therapy, recombination therapeutic proteins)	+	+/-[2]	+
Medical devices	+[3]	+/-[3]	+
Cell therapeutics	-	+/-	+

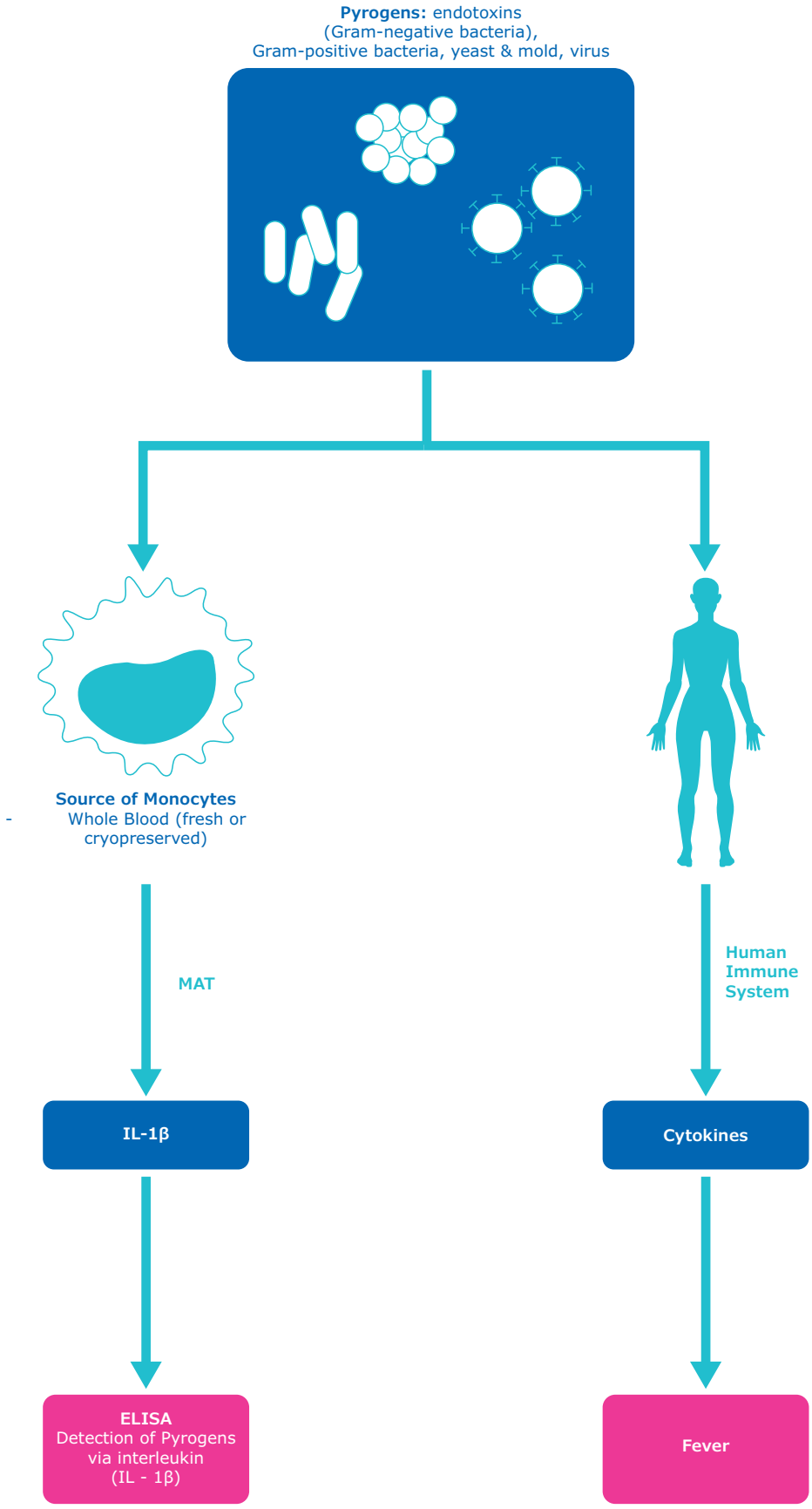
[1] Variable pyrogen results,

[2] Rabbit test often required,

[3] Indirect test with solution in pyrogen-free water

Comparison of the Rabbit Test, the LAL

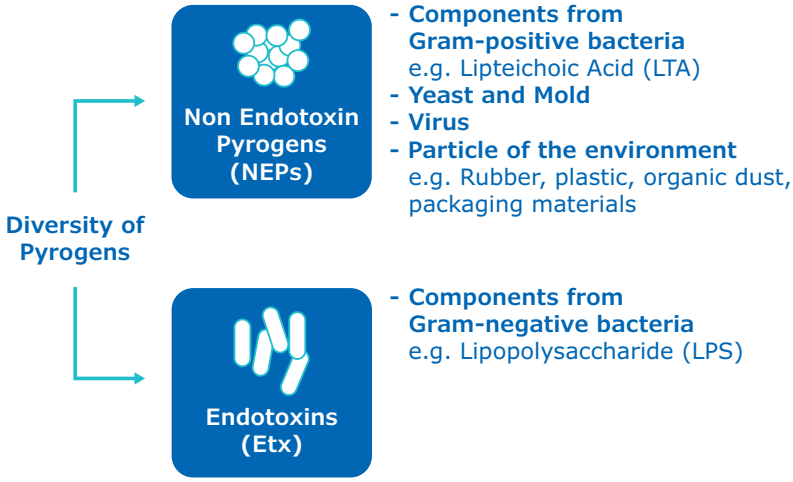
The PyroDetect System is a revolutionary test for the detection and quantification of pyrogens based on the Monocyte-Activation Test (MAT). The PyroDetect System mimics the innate immune defense reaction that happens in the human body when pyrogens are present in the blood stream. Monocytes activated by pyrogens produce cytokines that are detected in an immunological assay (ELISA) involving specific antibodies and an enzyme-mediated color change.



Broad pyrogen profile

Pryogens constitute a heterogeneous group of contaminants comprising microbial and non-microbial substances. The most widely known pyrogen is the endotoxin (LPS = Lipopolysaccharide), which is produced by gram-negative bacteria. Other microbial substances include those derived from gram-positive bacteria like the Lipoteichoic Acid (LTA), and particles from yeasts and viruses.

A source of non-microbial pyrogens is the production environment. Small particles from packaging, such as rubber or plastic, are also capable of causing fever in humans. For these reasons, it is extremely important to test for the full range of pyrogens within the pharmaceutical industry. The PyroDetect System covers a broad range of contaminations and applications, where both the LAL and Rabbit test are limited.



Limitation of the Rabbit and the LAL Test

	Rabbit	LAL
Products which can not be analysed	• Cytostatic drugs • Sedatives • Analgesics • Cytokines • Antibiotics • Chemotherapeutics • Proteins	• Blood products • Lipids • Celltherapeutics • Proteins
Controls	No	Yes
Animal consumption	++	+

Application limits for the Rabbit Test and the LAL Test.

Easy assay procedure

The PyroDetect System consists of three main steps, the cryoblood incubation, the IL-1β ELISA and the analysis.

1. Cryoblood incubation

The PyroDetect System uses cryoblood (pooled and frozen human whole blood) for the blood incubation. Fresh blood with a time limit of up to 4 hours after donation may also be used. The test samples are mixed with the blood in the cell culture plate and are kept in an incubator over night at 37 °C. If pyrogens are present in the sample, the monocytes of the blood will produce IL-1β during the incubation.

2. Interleukin-1β ELISA

For the detection of the IL-1β the cryoblood incubation mixture is transferred into an ELISA microplate coated with an antibody specific for IL-1β. Interleukin molecules present in the culture supernatant are bound by the immobilized antibody. Then an enzyme-linked polyclonal anti-body specific for IL-1β is added.

With the addition of the substrate a color reaction is started, which allows the detection of the bound IL-1β in an ELISA reader.

3. Analysis

The pyrogen concentration in the sample is then determined from the IL-1β concentration via an endotoxin standard curve. It is recommended to use a software for the analysis. Protocols to be used with Gen5 software from Biotek® are provided for free for the analysis of PyroDetect assay.

Assay procedure of the PyroDetect System

Robust application

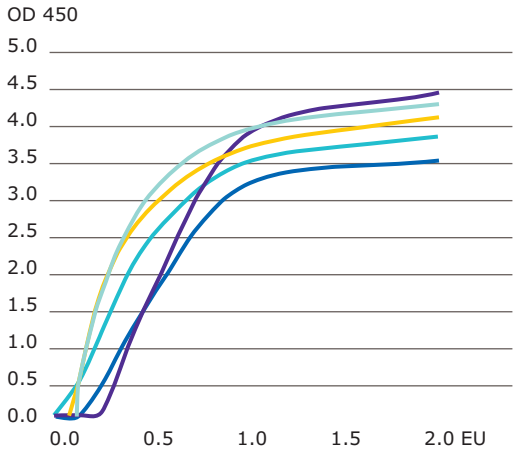
The PyroDetect System offers a high level of safety using both a positive and a negative control. The robust application of the test was shown in an international validation coordinated by the European Centre for the Validation of Alternative Methods (ECVAM) in 2005. Several published studies show the robustness and reliability of the test performance of the MAT.

The reproducibility of the PyroDetect System was shown in several user applications. The figure shows the results of five different users without routine experience in performing this kind of assays.

The PyroDetect System has a large pyrogen profile in the microbial field. Tests with Lipoteichoic Acid (LTA) indicate the detection of cell wall components of gram-positive bacteria with the PyroDetect System. As expected, the LAL test shows a reaction only to highly concentrated LTA. This is due to traces of endotoxin in LTA preparations. Thus the PyroDetect System reduces the security risk of non-endotoxin pyrogens and is an effective addition to the LAL test.

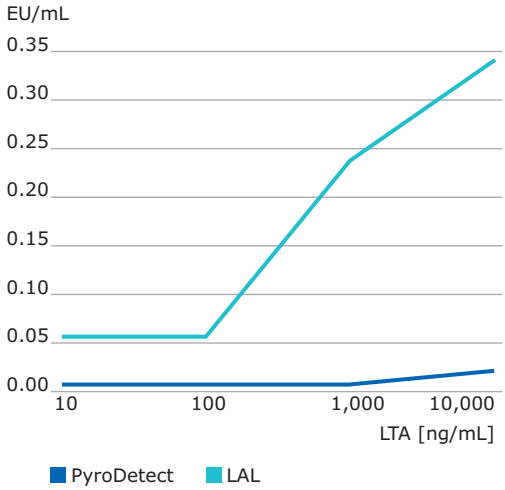
To ensure the detection of Non-Endotoxin Pyrogens (NEP) by the system, a positive control of NEP has been included in the Product Specific Validation protocol.

Reproducibility of standard curve



Comparable preparations of the endotoxin standard curve by five users. Different endotoxin dilutions are used in the PyroDetect assay procedure. Quantities of IL-1β are displayed as Optical Density (OD450) against the used concentrations (Endotoxin Units, EU).

Pyrogenicity of Lipoteichoic Acid (LTA)



The pyrogenic activity of Lipoteichoic Acid (LTA) is reliably detected with the PyroDetect System. Serial dilution of LTA in the PyroDetect System (red) as compared to the LAL Test (blue). Measured OD values are expressed as Endotoxin Units (EU) per mL referring to an international endotoxin standard.

Ordering information

The PyroDetect System consists of the PyroDetect Kit and the PyroDetect Cryoblood. Except for the endotoxin standard it contains all biological and biochemical reagents required for the MAT. To perform the test an endotoxin standard, the PyroDetect Endotoxin Standard, or an equivalent reference standard can be used.

PyroDetect System	Ord. No.
The main subsets of the PyroDetect System are:	
1. PyroDetect Kit Reagents for the cryoblood incubation and the Interleukin-1β ELISA Store at 2 – 8 °C	**1.44154.0001**
2. PyroDetect Cryoblood Cryoblood – deep frozen human whole blood preparation, 2 x 2 mL Store at -80 °C or lower	**1.44155.0001**
PyroDetect Endotoxin Standard International Reference Standard Endotoxin (RSE) – lyophilized Store at -20 °C, reconstituted at -40 °C	**1.44161.0001**
PyroDetect Interleukin Standard* Interleukin-1β Standard for control reactions in the Interleukin-1β ELISA Store at 2 – 8 °C	**1.44158.0001**

*To be used for the Product Specific Validation (check that the sample does not interfere with the detection system). Not needed for routine tests.

Required equipment for the PyroDetect System

Freezer (≤-80 °C) Storage of the PyroDetect Cryoblood
Incubator (37 °C) Temperature stabilization during the cryoblood incubation
ELISA reader (450 nm) Measurement of the Interleukin-1β concentration

The *in vitro* pyrogen test method with cryo-preserved blood is protected by US patent 9,784,753. EP 0 741 294 and EP 0 851 231. The ELISA part of this product is covered by one or more of the following U.S. patents held by R&D Systems, Inc. 614 McKinley Place NE Minneapolis, MN55413 USA.: 4,766,069; 5,510,462; 5,681,933; 4,762,914; 5,474,899; 5,789,185; 5,484,887; 5,122,459; 5,001,057; 5,077,219; 5,286,847.

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Filtration & Testing Products

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