

Pyrogenicity

Pyrogenicity is the capacity of a substance to induce a febrile (fever) response when introduced into the bloodstream, lymphatic system, or cerebrospinal fluid of an organism. The test methods below can be used to reliably assess a substance's pyrogenic potential and replace the rabbit pyrogen test (RPT) and the bacterial endotoxin test (BET)/limulus amoebocyte lysate (LAL) test.

	Method	Test Principle	Rationale
<i>In Vitro</i>	Monocyte Activation Test (MAT)	<ul style="list-style-type: none"> The MAT can detect both endotoxin and non-endotoxin pyrogens in a diverse set of products including pharmaceuticals, biologics, and medical devices, thus serving as a complete replacement for both the BET/LAL and RPT. The test system uses human monocytes from either whole blood, cryopreserved blood, peripheral blood mononuclear cells (PBMCs), or monocyte cell lines. The test sample is incubated with monocytes, and if pyrogens are present, monocytes are activated via toll-like receptors, triggering an immune response in the form of cytokine release. The MAT quantifies cytokine release from monocytes using ELISA. Cytokine concentrations are then compared to a standard curve to determine endotoxin equivalent unit concentration (EEU/mL), indicating the pyrogenic potential of the test sample. 	<ul style="list-style-type: none"> Uses human monocytes for pyrogen detection, providing direct relevance to human immune responses. The mechanism of monocyte activation and cytokine release mimics the human response. Delivers quantitative cytokine measurements by ELISA, eliminating the subjectivity of animal temperature readings. Produces results within hours after incubation instead of requiring days of animal monitoring. Offered as commercially available kits with built-in positive and negative controls to ensure reliable performance. Accepted by major global regulatory bodies as a valid method for detecting endotoxin and non-endotoxin pyrogens (replacing the RPT and BET/LAL).
<i>In Chemico</i>	Recombinant Factor C (rFC) Assay	<ul style="list-style-type: none"> The rFC is designed to only detect endotoxin pyrogens and serves as a complete replacement of the BET/LAL. The test system uses a reagent that contains recombinant Factor C and a quenched fluorogenic (or chemogenic) peptide substrate. The reagent is added to test samples and incubated. If endotoxins are present, they will bind and activate Factor C. Activated Factor C cleaves the substrate and releases a signal that is proportional to the concentration of endotoxins in the sample. This signal is read using a microplate reader and compared to a standard curve to determine endotoxin equivalent unit concentration (EEU/mL), indicating the pyrogenic potential of the test sample. 	<ul style="list-style-type: none"> Provides high-specificity endotoxin detection, avoiding interference from non-endotoxin contaminants. Uses a defined synthetic system with fully recombinant proteins to ensure batch-to-batch consistency and eliminate lot variability. Delivers quantitative fluorescence data via microplate reader analysis, replacing subjective clot assessment or animal-temperature measurements. Produces results with faster setup, less waste, and minimal variability when compared to the BET/LAL. Offered as validated, commercially available kits with built-in positive and negative controls to ensure reliable performance. Accepted by major global regulatory bodies as a valid method for detecting endotoxins (replacing the BET/LAL).

Guidance

- European Pharmacopoeia chapter 2.6.30, "Monocyte activation test. **As of July 1, 2025, mandated the transition from RPT to MAT. Must be validated for each product type.**
- European Pharmacopoeia chapter 2.6.32, "Test for bacterial endotoxins using recombinant Factor C." **As of July 1, 2020, rFC is recognized as an official method to detect endotoxins. Must be validated for each product type.**
- US Pharmacopoeia chapter <86>, "Bacterial Endotoxins Test Using Recombinant Reagents." **Allows for the use of rFC if validated for each product.**
- US Pharmacopoeia chapter <151>, "Pyrogen Test." **Allows the use of MAT if validated for each product.**
- The International Conference on Harmonisation. **Recommends that the analytical procedures described in the official pharmacopoeial texts, [European Pharmacopoeia] Ph.Eur. 2.6.14. Bacterial Endotoxins, [Japanese Pharmacopoeia] JP 4.01 Bacterial Endotoxins Test, and [United States Pharmacopoeia] USP General Chapter <85> Bacterial Endotoxins Test, can be used interchangeably in the ICH regions subject to the [specific] conditions.**
- US FDA's CDRH guidance (2020). **Formally recognizes rFC-based assays as acceptable for endotoxin testing with scientific justification and product-specific validation. Continues to support MAT with product-specific validation.**
- US FDA's Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers (2012). **States that alternatives, specifically the MAT, may be used after product-specific validation.**
- ISO 10993-1:2018 "Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process." **Gives preference to in vitro models when they yield equally relevant information.**

Many companies offer MAT kits. For more information, please see ThePSCI.eu/our-work/pyrogenicity