A complete solution for GLP/GMP-compliant endotoxin testing: from raw data to reliable result

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Introduction

Recent revisions to the European Pharmacopoeia have accelerated the transition toward modern, sustainable and animal-free pyrogen and endotoxin testing,. Molecular Devices provides a comprehensive workflow for endotoxin detection, combining SpectraMax® microplate readers with SoftMax® Pro Software for data acquisition, analysis, reporting and assay-specific calculations, supporting kinetic chromogenic and turbidimetric LAL assays, rFC assays and the Monocyte Activation Test. This poster focuses on the animal-free methods: the MAT and the PyroGene® Recombinant Factor (rFC) endotoxin detection, combining assay performance data with the assay-specific workflows and instrument configurations.

Comparison of pyrogen and bacterial endotoxin test methods and compatible SpectraMax microplate readers

	Pyrogen Tests	Bacterial Endotoxin Tests	
	MAT	LAL: Chromogenic and Turbidimetric	rFC
Detects endotoxins	✓	✓	✓
Detects non-endotoxin pyrogens	✓		
Animal-free	✓		✓
Regulatory acceptance	Ph. Eur. 2.6.30	Ph. Eur. 2.6.14USP <85>	Ph. Eur. 2.6.32USP <86>
Reading setting	Absorbance, endpoint, 405 nm	Absorbance, Kinetic, Chromogenic: 405 nm Turbidimetric: 340 nm, slow carrier speed	Fluorescence: endpoint or kinetic, Ex: 380 nm, Em: 440 nm
Temperature	Ambient	37°C	37°C
SpectraMax ABS / ABS Plus	✓	✓	
SpectraMax i-Series	✓	✓	✓
SpectraMax M-Series	✓	✓	✓

GxP compliance solutions for regulated workflows

GxP Compliance Solutions include a combination of software, hardware, and services:

- **SoftMax® Pro GxP Software:** delivers FDA 21 CFR Part 11 compliance tools with secure user access, electronic records, e-signatures, and comprehensive audit trails to ensures data integrity and traceability in regulated workflows.
- **Microplate readers and washers:** designed to operate within validated laboratory environments.
- **IQ/OQ/PQ and documentation services:** support instrument setup and verification in regulated laboratories.
- **Software installation and qualification services:** verify that required software components are installed and configured according to defined specifications
- **SpectraTest® validation plates and recertification services:** verification of microplate reader performance.

1 Plate preparation & incubation — Endotoxin assay kit
- Standards and controls
- Samples and PPCs
- Kit-specific plate layout
- Incubation conditions

Preparation

2 Plate read — SpectraMax® Reader
- Absorbance or fluorescence
- Endpoint or kinetic read
- Assay-specific settings including temperature

Acquisition

3 Data analysis — SoftMax® Pro GxP Software
- 23 Curve fits including linear and 4PL / 5PL
- Parallel Line Analysis (PLA)
- Goodness of Fit and Lack of Fit

Analysis

4 Reporting results — SoftMax® Pro GxP Software
- Calculated sample results
- Assay specific validity tests: Pass / fail evaluation
- Standardized report output

Reporting

5 Data integrity and management — SoftMax® Pro GxP Software
- User access and permissions
- Audit trail
- Electronic records
- Archiving and traceability

Controlled records and traceability

Data analysis and reporting

Custom SoftMax® Pro protocols have been developed to meet the assay requirements and perform the intermediate calculations required to generate the output parameters for all endotoxin assays. These calculations can be further adapted to meet kit-specific or laboratory-specific requirements. Final results, including the pass/fail outcome for each test are summarized in the conclusion without further user intervention.

MAT

The MAT assay is performed using an endpoint absorbance detection at 405 nm as recommended for the MAT Research assay workflow. The custom protocol is composed of two sections:

- **MAT_MATResearch:** contains product specifications, acceptance criteria, information to enter or verify, probability calculations for lack-of-fit and regression testing, result interpretation, formulas, and test conclusions for the standard group (RSE_curve).
- **Expt1:** contains the plate section for data acquisition, analysis tables, and graph section.

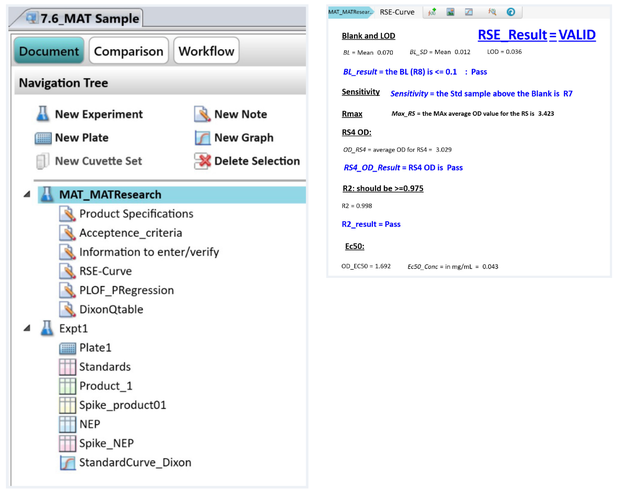


Figure 1. *5 PL MAT assay standard curve.*





Recombinant Factor C (rFC) Assay

The rFC Assay measures the activation of recombinant Factor C using a fluorogenic substrate. The plate is incubated in the instrument for one hour at 37°C and read using a simple workflow, followed by automated endotoxin quantification using a log-log standard curve in SoftMax® Pro Software.

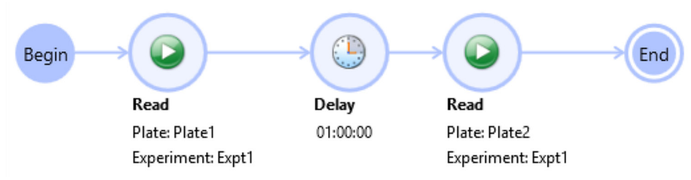
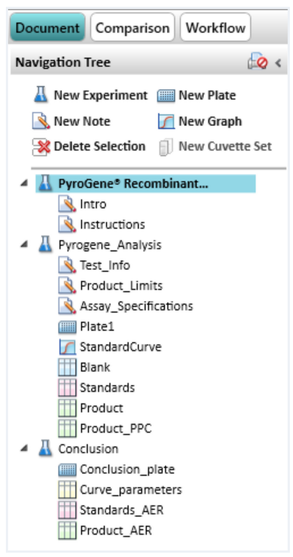


Figure 2. *Workflow setup in SoftMax Pro Software.*

The custom protocol is composed of 3 sections:

- **PyroGene® Recombinant Factor C:** introduction and instructions.
- **Pyrogene_Analysis:** contains the plate section, analysis tables, and graph section, as well as the assay acceptance criteria and information on the specific run.
- **Conclusion:** contains test conclusions for the curve, Standards and Product groups.



Sample	Dilution	CVs	Endotoxin_ EU/mL	CV_ Specification	Status	Quantification_ alert
01	1.0	N/A	< 0.005	< 25 %	Not Calculated	Undefined
02	2.0	N/A	< 0.005	< 25 %	Not Calculated	< LS
03	1.0	1.2	0.05557	< 25 %	PASS	N/A
04	1.0	3.4	0.69579	< 25 %	PASS	N/A
05	1.0	0.7	> 5.000	< 25 %	PASS	N/A

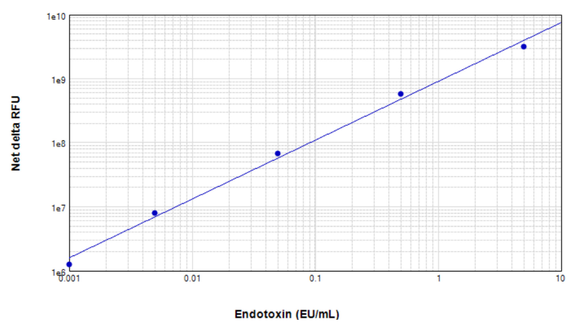


Figure 3. *PyroGene rFC assay standard curve.*

Conclusion

One platform covers pyrogen and endotoxin assays: MAT, rFC and the chromogenic and turbidimetric LAL assays. Ready-to-use SoftMax Pro protocols automate curve fitting, goodness-of-fit and lack-of-fit testing, acceptance criteria and pass/fail reporting, so no manual calculation is required. Combined with GxP software, qualified SpectraMax readers and IQ/OQ/PQ services, this helps implementation and validation for regulated laboratories.

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