PYROLAB
Monocyte Activation Test (MAT) Kit
Validation Guide

Catalog No.: KBPL9110

Kit Format	Monocyte Activation Test (MAT); absorbance readout at 450 nm
Calibration Range (Linearity)	0.0125 – 1 EU/mL
Quantitative Range	0.025 – 0.6 EU/mL
Quantitation Limit (QL)	0.025 EU/mL
Detection Limit (DL)	0.0125 EU/mL
Manufacturing	ISO 13485
Regulatory Reference	Ph.Eur. <2.6.30>, ICH Q2(R2), ChP <9101>, <9301>, <9401>

1. Introduction

The PYROLAB Monocyte Activation Test (MAT) Kit is an in vitro assay for the quantitative determination of pyrogenic activity, expressed in endotoxin equivalents (EU/mL). Unlike the LAL test, the MAT responds to both endotoxin and non-endotoxin pyrogens (NEPs).

This Validation Guide summarises assay performance data for the kit, evaluated with reference to:

- ICH Q2(R2) – Validation of Analytical Procedures
- Ph.Eur. <2.6.30> – Monocyte-activation Test

Parameters evaluated: linearity, range, quantitation limit (QL), detection limit (DL), repeatability, accuracy, intermediate precision, specificity (endotoxin-related specificity, NEP detection and sample interference) and robustness (instrument suitability and lot-to-lot consistency).

2. Kit Overview & Intended Use

Parameter	Details
Kit Name	PYROLAB Monocyte Activation Test (MAT) Kit
Catalog Number	KBPL9110
Assay Format	Monocyte Activation Test (MAT); $OD_{450\ nm}$ readout
Calibration Range (Linearity)	0.0125 – 1 EU/mL
Quantitative Range	0.025 – 0.6 EU/mL
Quantitation Limit (QL)	0.025 EU/mL
Detection Limit (DL)	0.0125 EU/mL
Pyrogens Detected	Endotoxin; non-endotoxin pyrogens (lipoteichoic acid, resiquimod)
Microplate Readers Tested	Thermo Multiskan FC; MD SpectraMax M2
Manufacturing Standard	ISO 13485
Intended Use	Research use only; not for use in diagnostic procedures or for therapeutic purposes in humans or animals

3. Linearity and Range

Linearity of the standard curve was assessed across 0.0125 – 1 EU/mL in five independent tests. R^2 was evaluated for each test.

3.1 Acceptance Criteria

- $R^2 \geq 0.980$ for all tests

3.2 Linearity Data Table

Standard curve parameter	Test-1	Test-2	Test-3	Test-4	Test-5
R^2	0.999	0.997	0.999	0.999	0.997
Acceptance criteria	$R^2 \geq 0.980$				
Result	**Pass**	**Pass**	**Pass**	**Pass**	**Pass**

4. Quantitation and Detection Limits

4.1 Quantitation Limit (QL)

The QL is the lowest concentration quantified with acceptable precision and accuracy. Acceptance: upper limit of the 95% confidence interval of RSD ≤ 30%, and 95% confidence interval of recovery within 70% – 130%. Two low concentrations were tested (n = 18 each). The QL of the assay is **0.025 EU/mL**.

Theoretical Conc. (EU/mL)	0.025 (n=18)	0.0125 (n=18)
Ave. value	0.024	0.016
Upper limit of 95% CI of RSD (%)	12.42	10.68
Lower limit of 95% CI of recovery (%)	91.80	126.33
Upper limit of 95% CI of recovery (%)	100.28	136.29

4.2 Detection Limit (DL)

The DL is the lowest concentration point in the four-parameter fitted standard curve at which the average $OD_{450\ nm}$ of the detection-limit sample exceeds the threshold (average $OD_{450\ nm}$ of the negative control + 3SD). The DL of the kit is **0.0125 EU/mL**, confirmed in three independent experiments.

Experiment	0.0125 EU/mL Ave. $OD_{450\ nm}$ (n=4)	Negative control Ave. $OD_{450\ nm}$ (n=4)	Threshold (Neg. ctrl + 3SD)	DL (EU/mL)
1	0.092	0.056	0.066	0.0125
2	0.092	0.067	0.072	0.0125
3	0.146	0.074	0.134	0.0125

5. Range, Accuracy and Precision

Quality control samples (QCS) were prepared at six concentration levels within the calibration range: 1, 0.6, 0.4, 0.2, 0.04 and 0.025 EU/mL (n = 6 each).

5.1 Repeatability

Acceptance: upper limit of the 95% confidence interval of RSD ≤ 30% at all levels.

Theoretical Conc. (EU/mL)	1 (n=6)	0.6 (n=6)	0.4 (n=6)	0.2 (n=6)	0.04 (n=6)	0.025 (n=6)
Ave. value (EU/mL)	0.824	0.560	0.384	0.223	0.037	0.025
Upper limit of 95% CI of RSD (%)	21.6	22.7	19.9	6.8	6.5	13.6

5.2 Range, Accuracy and Intermediate Precision

Acceptance: upper limit of the 95% confidence interval of RSD ≤ 30% and 95% confidence interval of recovery within 50% – 200%. All levels from 0.025 to 0.6 EU/mL met the requirements for accuracy and intermediate precision. The 1 EU/mL level did not meet the RSD criterion (47.2%), so the quantitative range of the kit is **0.025 – 0.6 EU/mL**.

Theoretical Conc. (EU/mL)	1 (n=6)	0.6 (n=6)	0.4 (n=6)	0.2 (n=6)	0.04 (n=6)	0.025 (n=6)
Ave. value (EU/mL)	0.903	0.551	0.446	0.219	0.041	0.025
Upper limit of 95% CI of RSD (%)	47.2	16.0	15.1	18.0	22.2	16.2
Lower limit of 95% CI of recovery (%)	68.9	84.4	103.0	99.4	90.3	93.6
Upper limit of 95% CI of recovery (%)	111.7	99.2	119.8	119.1	112.9	110.1

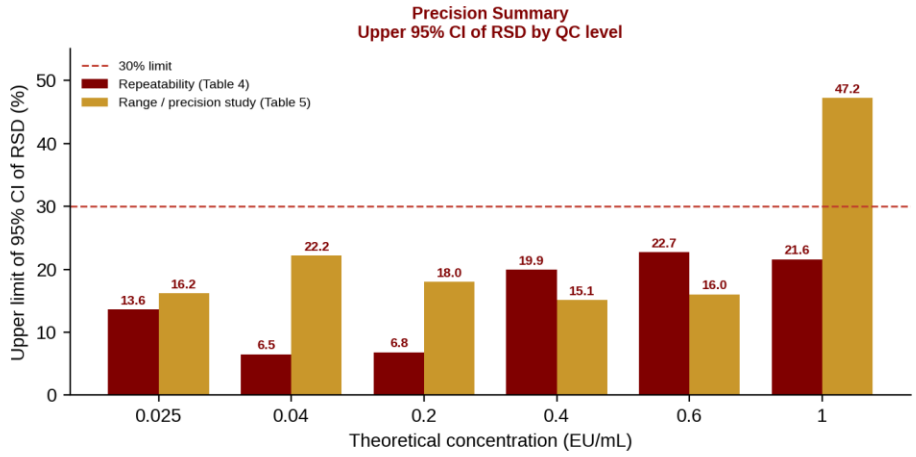


Figure 1. Upper limit of the 95% CI of RSD for repeatability (maroon) and the range/precision study (gold). Red dashed line = 30% limit.

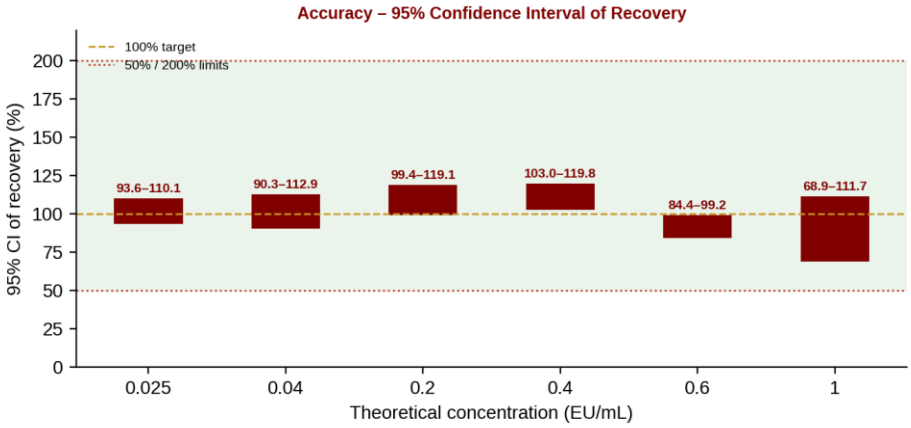


Figure 2. 95% confidence interval of recovery at each QC level. Shaded zone = 50–200% acceptance range; gold dashed line = 100% target.

6. Specificity

6.1 Endotoxin-Related Specificity

A 0.1 EU/mL endotoxin standard was heat-inactivated (inactivated group) and compared with a non-inactivated control group. The control group was quantified normally (121.0% recovery), whereas the inactivated group showed markedly reduced detection (15.0% recovery). The kit therefore accurately quantifies active endotoxin but not inactivated endotoxin, confirming that the signal depends on endotoxin activity.

Group	Ave. $OD_{450\ nm}$	Ave. value (EU/mL)	Recovery (%)
Control group	0.933	0.121	121.0
Inactivated group	0.069	0.015	15.0

6.2 Detection of Non-Endotoxin Pyrogens (NEP)

The kit detects at least two non-endotoxin pyrogens, lipoteichoic acid (LTA) and resiquimod. Acceptance: each NEP sample has at least one concentration above the QL (0.025 EU/mL).

Sample	Conc. (μg/mL)	Ave. value (EU/mL)	Result
Lipoteichoic acid (LTA)	0.1	0.022	**Pass**
	1	0.041	
	10	0.842	
Resiquimod	1	0.018	**Pass**
	10	2.762	
	50	>2.762	

6.3 Interference Test

Normal saline for injection was used as the sample matrix. Acceptance: the negative (unspiked) sample result is below the QL, and spike recovery is within 50% – 200%. Spike recoveries of 92.0% – 100.0% were obtained at 10-, 20- and 40-fold dilution, indicating that the sample matrix did not interfere with the assay.

Sample	Dilution multiple	Ave. value (EU/mL)	Spiked ave. value (EU/mL)	Recovery (%)
Normal saline for injection	10	0.013	0.113	100
	20	0.013	0.112	99
	40	0.014	0.106	92

7. Robustness

7.1 Instrument Suitability

Instrument suitability was tested on a Thermo Multiskan FC and an MD SpectraMax M2 at 0.025 EU/mL (QL) and 0.05 EU/mL (2 × QL). Acceptance: upper limit of the 95% CI of RSD ≤ 30% and 95% CI of recovery within 70% – 130%. Both readers met the requirements.

Microplate reader	Thermo Multiskan FC		MD SpectraMax M2	
Theoretical conc. (EU/mL)	0.025 (n=12)	0.05 (n=12)	0.025 (n=12)	0.05 (n=12)
Ave. value (EU/mL)	0.023	0.048	0.026	0.054
Upper limit of 95% CI of RSD (%)	15.0	14.4	19.7	22.8
Lower limit of 95% CI of recovery (%)	87.8	89.8	96.2	97.3
Upper limit of 95% CI of recovery (%)	99.3	101.1	113.1	117.3

7.2 Lot-to-Lot Consistency

Two kit lots were tested at 0.025 EU/mL (QL) and 0.05 EU/mL (2 × QL) using the same criteria (RSD ≤ 30%; recovery 70% – 130%). Both lots met the requirements for batch consistency.

Batch No.	Batch 1		Batch 2	
Theoretical conc. (EU/mL)	0.025 (n=12)	0.05 (n=12)	0.025 (n=12)	0.05 (n=12)
Ave. value (EU/mL)	0.022	0.048	0.027	0.054
Upper limit of 95% CI of RSD (%)	10.5	17.1	13.9	20.6
Lower limit of 95% CI of recovery (%)	85.8	88.3	102.4	98.7
Upper limit of 95% CI of recovery (%)	93.6	101.7	114.7	116.9

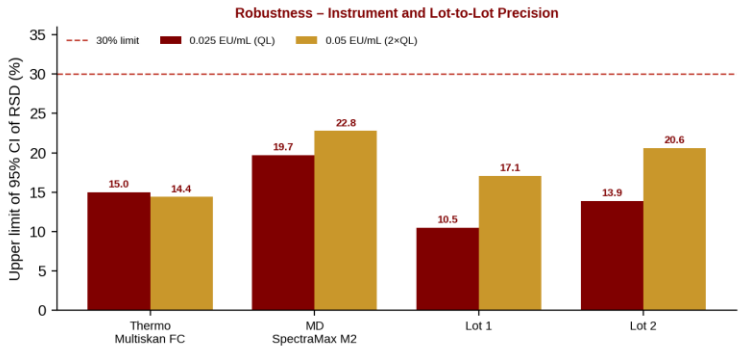


Figure 3. Upper limit of the 95% CI of RSD for instrument suitability and lot-to-lot consistency. Red dashed line = 30% limit.

8. Validation Summary

Parameter	Result
Linearity (R^2)	0.997 – 0.999 across 5 tests (0.0125 – 1 EU/mL)
Quantitation limit (QL)	0.025 EU/mL; RSD ≤ 12.42%; recovery 91.80 – 100.28%
Detection limit (DL)	0.0125 EU/mL (3 of 3 experiments)
Repeatability	Upper 95% CI of RSD 6.5% – 22.7% (all ≤ 30%)
Range / accuracy / precision	0.025 – 0.6 EU/mL: RSD ≤ 22.2%; recovery 84.4 – 119.8%
Upper standard (1 EU/mL)	Upper 95% CI of RSD 47.2% (> 30%)
Endotoxin specificity	Active endotoxin 121.0%; inactivated 15.0%
NEP detection	LTA and resiquimod detected above QL
Interference (saline)	Recovery 92.0 – 100.0% at 10–40× dilution
Instrument suitability	2 readers: RSD ≤ 22.8%; recovery 87.8 – 117.3%
Lot-to-lot consistency	2 lots: RSD ≤ 20.6%; recovery 85.8 – 116.9%

All evaluated parameters met their predefined acceptance criteria within the claimed quantitative range of 0.025 – 0.6 EU/mL, with a detection limit of 0.0125 EU/mL and a linear calibration range of 0.0125 – 1 EU/mL. The PYROLAB Monocyte Activation Test (MAT) Kit is validated for quantitation of pyrogenic activity across 0.025 – 0.6 EU/mL, in accordance with the principles of ICH Q2(R2) and Ph.Eur. <2.6.30>. Suitability must be confirmed with the user's own samples.

9. References

1. ICH Q2(R2): Validation of Analytical Procedures
2. European Pharmacopoeia (Ph.Eur.): <2.6.30> Monocyte-activation Test