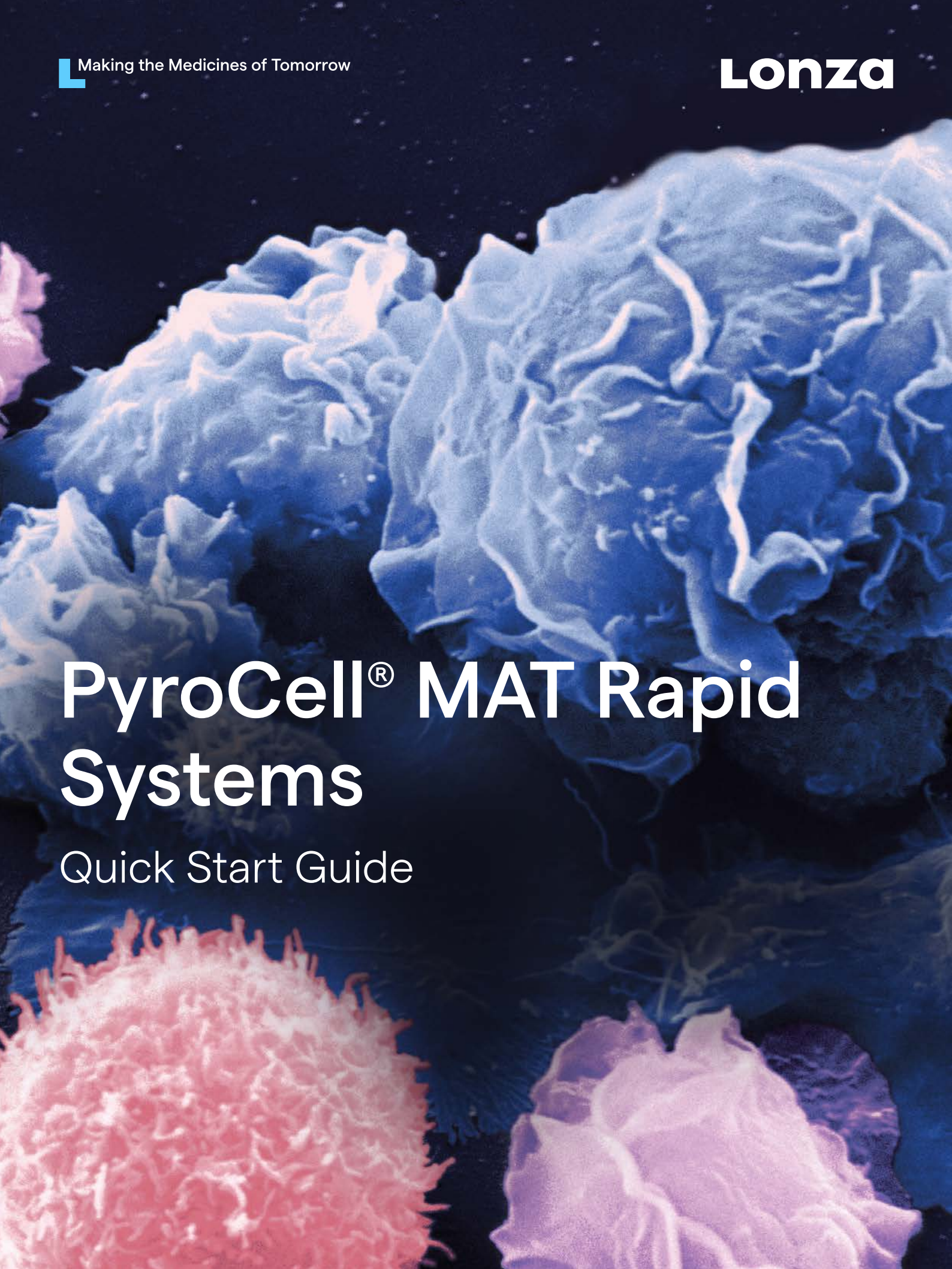
Making the Medicines of Tomorrow

Lonza



PyroCell® MAT Rapid Systems

Quick Start Guide

Routine Use of the PyroCell® MAT Rapid Systems Sustainable, *In Vitro* Detection of Pyrogens

PyroCell® MAT Rapid Systems comprise cryopreserved PBMC pooled from 4 qualified human donors (pMAT cells), an optimized Culture Medium Supplement and the PeliKine Human IL-6 Rapid ELISA Kit. A choice of 2 supplements, fetal bovine serum (FBS), or human serum (HS) allows you to choose the best solution for your testing needs.

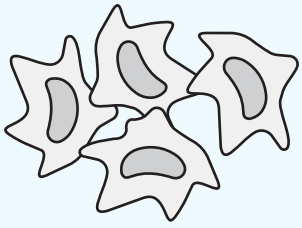
Overview

Monocytes, the key cells of innate immunity, respond to pyrogens in a test substance by releasing pro-inflammatory cytokines during a stimulation step. Following, the IL-6 cytokines contained in the cell culture supernatants are detected with the Pelikine Human IL-6 Rapid ELISA and compared to IL-6 release stimulated by reference standard.

Regulatory requirements as outlined for example, by the European Pharmacopeia (Ph. Eur 11.5 Chapter 2.6.30) describe the following MAT methods: Method 1 (semi-quantitative test) and Method 2 (reference lot comparison test). This document describes an example employing Method 1. Test sensitivities for the PyroCell® MAT Rapid Systems are ≤ 0.02 for the FBS-based assays and ≤ 0.08 for the HS-based assay. For more details of the test procedure please refer to the respective PyroCell® MAT Rapid System User Guide.

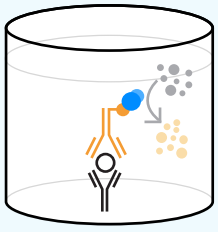
Day 1: **Stimulation of pMAT Cells**

- Prepare complete medium, reference dilutions, and test samples
- Allocate all dilutions on a 96-well cell culture plate
- Thaw pMAT Cells, transfer to the plate and incubate for 18 – 24h

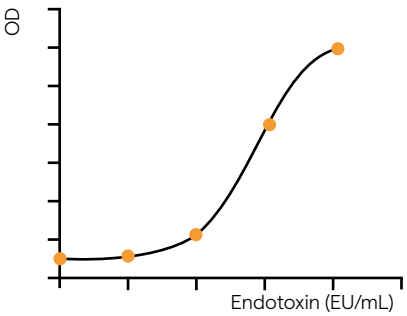


Day 2: **IL-6 ELISA**

- Harvest the culture supernatants
- Detect IL-6 cytokines with the IL-6 Rapid ELISA
- Read results in a Microplate absorbance reader



Calculate the pyrogenicity of the test sample.



Day 1: Stimulation of pMAT Cells

Prepare complete medium, reference dilutions, test samples and optional controls

Points to consider

- **Do not vortex dilutions containing complete medium or pMAT Cells.**
- Prepare all dilutions for cell culture in an aseptic environment (laminar airflow cabinet) at the day of experiment.
- Use pyrogen-free accessories and tubes only. Equilibrate reagents to room temperature before use.
- Avoid bubbles and foam formation.
- Do not exceed the maximum valid dilution (MVD).

	PyroCell® MAT Rapid Kit, Cat. No. 296407		PyroCell® MAT HS Rapid Kit, Cat No. 296408
Complete Medium (CM)			
	33 mL IMDM + 1 vial FBS supplement		38 mL IMDM + 1 vial HS supplement
Endotoxin Standard Curve			
Reference points	5	7	7
Concentrations EU/mL	0.16 – 0.08 – 0.04 – 0.02 – 0.01 – Blank	0.64 – 0.32 –0.16 – 0.08 – 0.04 – 0.02 – 0.01 – Blank	1.28 – 0.64 – 0.32 – 0.16 – 0.08 – 0.04 – 0.02 – Blank
Prepare Endotoxin Solutions			
• Stock solution 2,200 EU/mL	Reconstitute RSE (# E700) with 5 mL of LAL Reagent Water (# W50-100) according to vendor instructions. Alternatively, thaw a 50 µL aliquot and vortex for three minutes.		
• Serial dilution #1 → 100 EU/mL	Dilute 45.5 µL stock solution in 954.5 µL IMDM medium (#12-722F)		
• Serial dilution #2 → 4 EU/mL	Dilute 40 µL 100 EU/ mL in 960 µL complete medium (CM). Do NOT vortex!		
Endotoxin work concentration (EU/mL): 1 mL	0.16 EU CM: 960 µL, RSE 4 EU: 40 µL	0.64 EU CM: 840 µL, RSE 4 EU: 160 µL	1.28 EU CM: 680 µL, RSE 4 EU: 320 µL
RSE spike solution (PPC): 2 mL	0.16 EU/mL CM: 80 µL, RSE 4 EU: 1920 µL	0.16 EU CM: 1920 µL, RSE 4 EU: 80 µL	0.32 EU CM: 1840 µL RSE 4 EU: 160 µL
Test Sample and optional controls			
Test sample dilution: 2.5 mL	Optimum sample dilution from preparatory testing		
Optional NEP spike: 1 mL	Spike into optimum sample dilution, demonstrate spike recovery 50 – 200%		
Optional: IL-6 standard curve	Leave space empty, add IL-6 standard dilutions to ELISA plate		

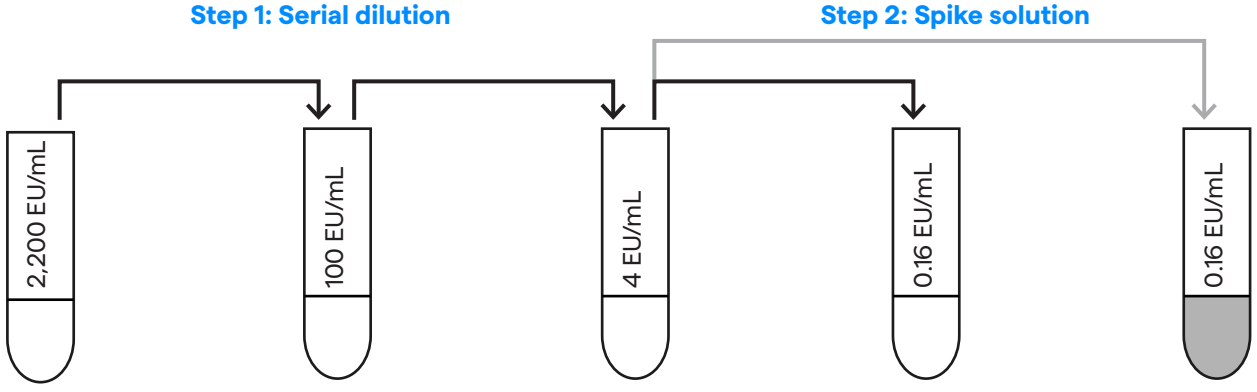
For more detailed instructions please refer to the User Manual

Note: The RSE stock solution stated above and in the diagram below are for USP lot R172R0.

Endotoxin Dilutions

In this example,

- Prepare complete medium by adding MAT Culture Medium Supplement to 33 mL IMDM. Mix by inverting the tube 10x.



RSE stock solution in LAL water

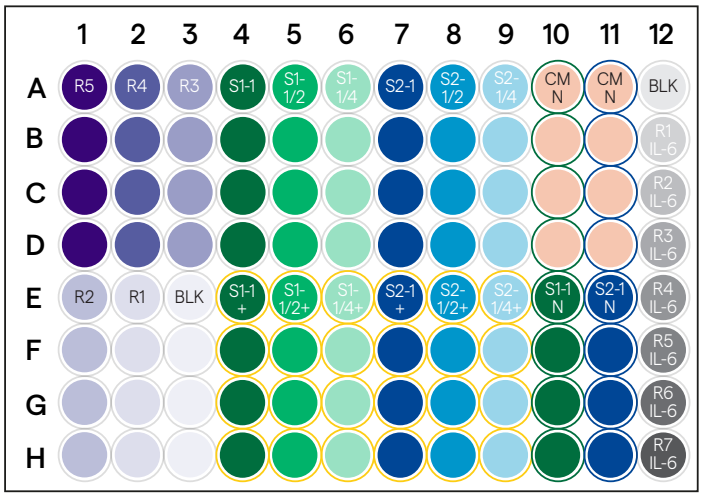
45.5 µL RSE dilution, 954.5 µL IMDM

40 µL RSE dilution, 960 µL complete medium

40 µL RSE dilution, 960 µL complete medium

80 µL RSE solution 1920 µL complete medium

Overview Plate Layout, Example



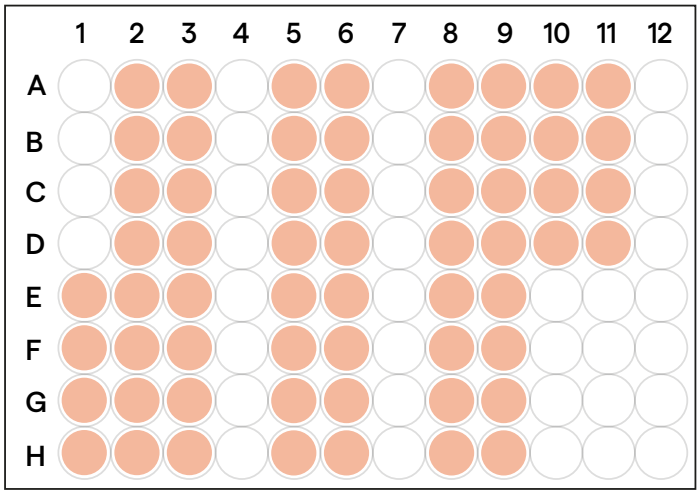
Column 1 - 3: 5-point standard curve and a blank (purple)

Column 4 - 6 and 7 - 9: test sample #1 and #2 with and without control endotoxin spike (+, green and blue, spike yellow circle)

Column 10 - 11: NEP spike into complete medium (CM N) and samples (N, blue circle)

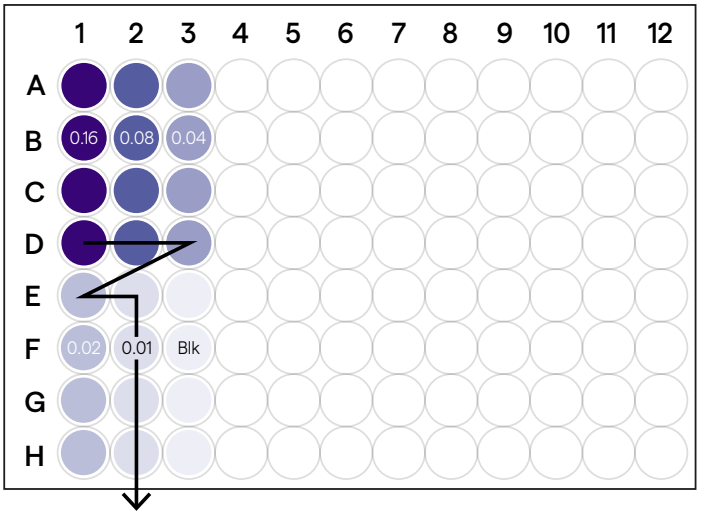
Column 12: Blank space for optional IL-6 standard curve during ELISA

Prepare the Cell Culture Plate



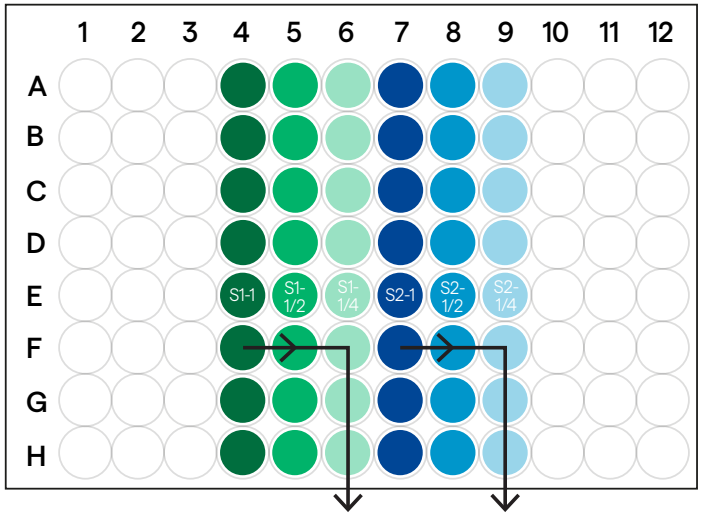
3: Transfer complete medium

- Add 100 µL of complete medium into every orange well.



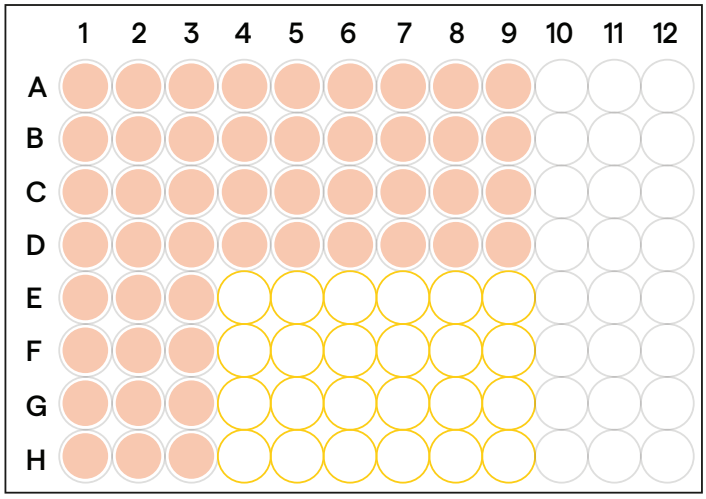
4: Prepare the endotoxin standard curve

- Add 200 µL of the 0.16 EU/mL RSE dilution into wells A1 – D1.
- Transfer 100 µL from A1 – D1 to A2 – D2. Mix by pipetting up and down 10x.
- Repeat transfer from A2 – D2 to A3 – D3, then to E1 – H1 and E2 – H_2 as illustrated.
- Discard 100 µL from E2 – H_2.



5: Prepare the sample dilutions

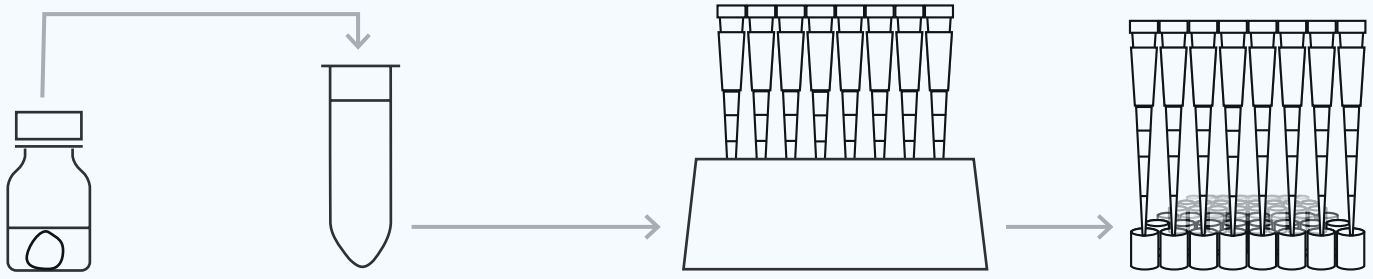
- Add 200 µL of the sample S1 into column 4.
- Transfer 100 µL from column 4 to 5, and column 5 to 6. Mix each time by pipetting up and down 10x.
- Discard 100 µL from column 6.
- Repeat 2-fold dilution for other test samples, e.g. sample S2 (columns 7 – 9).
- (Optional for NEP) Add 100 µL of sample S1 into E10 – H10 and sample S2 into E11 – H11.



6: Add endotoxin spike and optional, NEP control

- Add 50 µL of complete medium into light orange wells.
- Add 50 µL of endotoxin spike solution (0.08 EU/mL) to the yellow circles.
- Add 50 µL of NEP to column 10 and 11.

Thaw pMAT Cells, Transfer to Plate and Incubate



Step 1
Thaw a vial of pMAT Cells at 37°C until observing a small remaining clump of ice.

Step 2
Immediately transfer thawed cells into a 50 mL tube and slowly add 5 mL of complete medium while swirling gently. **Do not vortex!**

Step 3
Fill cell suspension into a reservoir.

Step 4
Transfer 50 µL pMAT Cells to each well of columns 1 – 11; incubate the plate at 37°C for 18 – 24 hours.

Day 2: IL-6 ELISA Assay

Points to consider

- During wash steps, make sure to empty all wells completely after the last wash step.
- Equilibrate all buffers and solutions to room temperature prior to use (exception: anti-IL 6 antibody and streptavidin-HRP reagent).

1: Buffer Preparation

Buffer	Concentrate	dH_2O
Wash buffer	50 mL	950 mL
HPE buffer	15 mL	60mL

2: "Sandwich ELISA"

- Transfer supernatants from the cell culture plate (day 1) to a fresh plate. Keep the original layout
- Homogenize supern atants in fresh plate by pipetting up and down
- Add 20 µL of supernatant of each well to the pre-coated plate
- Dilute 120 µL of biotinylated anti-IL-6 antibody in 9.6 mL HPE buffer. Add 80 µL to each well of the pre-coated plate

Binding the HRP conjugate

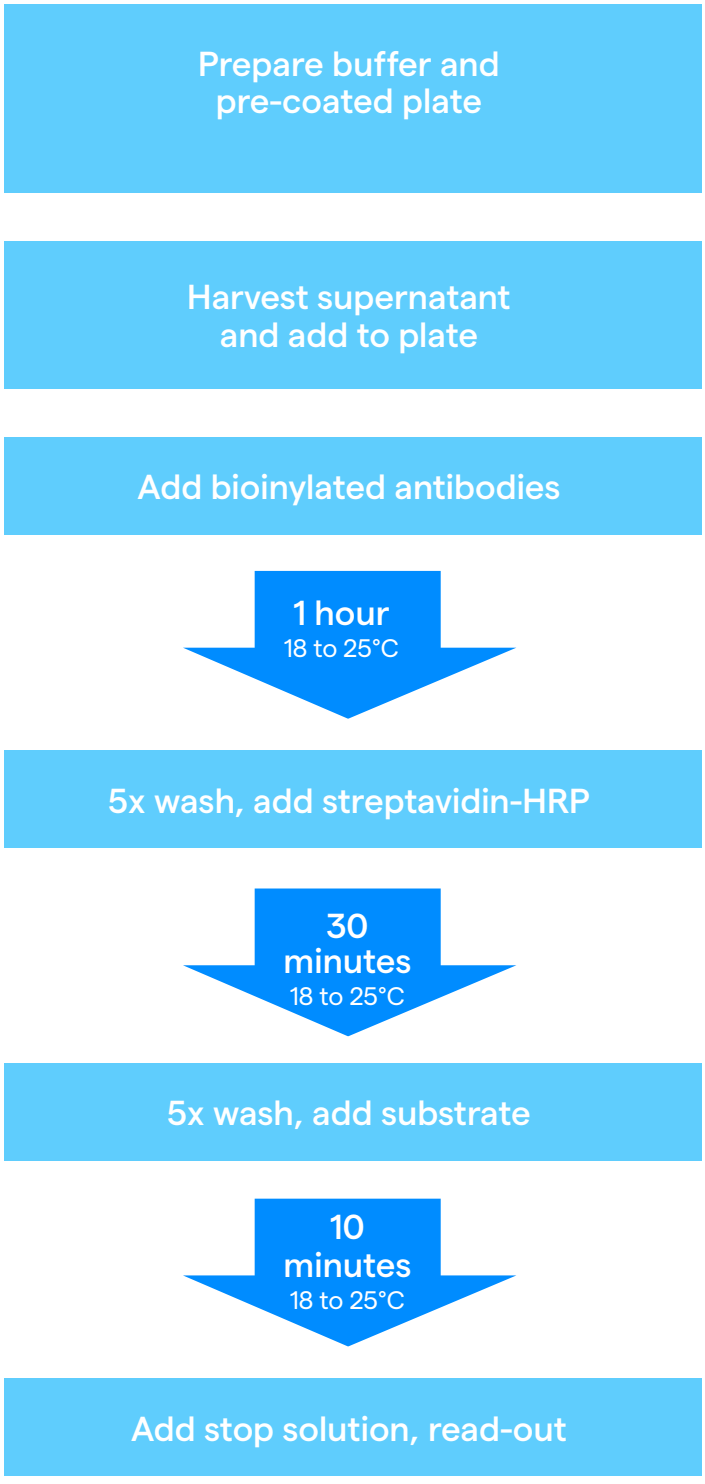
- Remove contents. Wash plate 5x with 300 µL/well wash buffer
- Dilute 2 µL of Streptavidin antibody in 20 mL HPE buffer. Add 100 µL to each well of the pre-coated plate

Color reaction

- Remove contents. Wash plate 5x with 300 µL/well wash buffer
- Add 100 µL TMB substrate

3: Reading results

- Add 100 µL Stop Solution
- Read absorbance (OD) at OD450 nm and a reference wavelength (OD540-590 nm) in a plate reader (e.g. Nebula® System) and analyze results



MAT Reagents and Pyrogen-free Materials

Product name	Cat. no.
PyroCell® MAT Rapid System	00296407
PyroCell® MAT HS Rapid System	00296408
Iscove's Modified Dulbecco's Medium(IMDM)	12-722F
Reference Standard Endotoxin (RSE – USP)	E700
LAL Reagent Water (e.g. 100 mL)	W50-100
Sterile, pyrogen-free glass tubes	N207
Reagent Reservoir	00190035
LAL Reagent Grade™ Multi-well Plates	25-340
Pyrogen-free Eppendorf® Biopur® Tips, 2 – 200 µL	25-415
Pyrogen-free Eppendorf® Biopur® Tips, 50 – 1000 µL	25-417
Nebula® Absorbance Reader	25-365S
Nebula® Multimode Reader	25-375S
(optional) Non-endotoxin pyrogens	

What You Also Need

- 50 mL tubes (cell-culture grade, endotoxin-free)
- Bottles and test tubes (buffer preparation)
- Volumetric and serological pipette (as needed)
- Adjustable pipettes, multichannel pipette
- Pipette tips (standard)
- 96-well round bottom plates (standard)
- Laminar airflow cabinet (aseptic)
- CO_2 cell incubator (37°C, 5% CO_2)
- Water bath (37°C)
- Automated microplate washer and microplate shaker

Supporting Resources

- Certificate of Analysis (CoA), www.lonza.com/coa
- PyroCell® MAT Rapid System User Guides for Routine Use
- PyroCell® MAT Analytics Templates
- **eLearning Training On-demand** – Please contact us

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