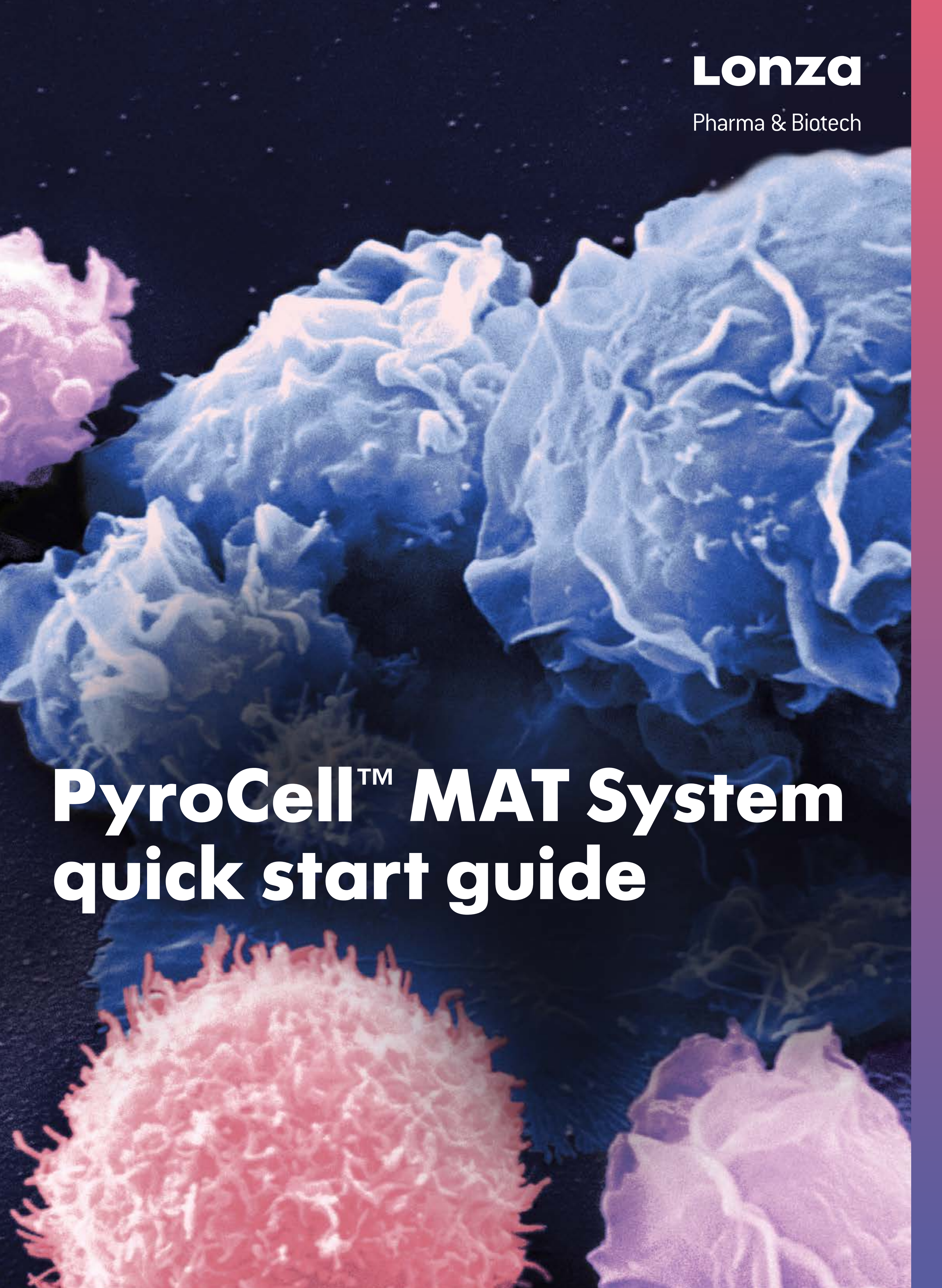

PyroCell™ MAT System quick start guide

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Routine use of the PyroCell™ MAT System for *in vitro* detection of pyrogenic contaminants in test samples

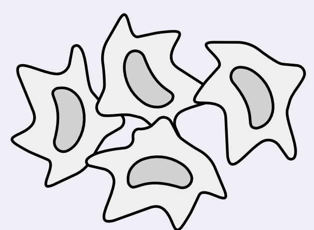
The PyroCell™ MAT System, IL-6 comprises cryopreserved PBMC pooled from 4 healthy human donors (pMAT Cells), an optimized MAT Culture Medium Supplement, and a human IL-6 ELISA assay system (00254714). Monocytes, the key cells of innate immunity, respond to pyrogens in a product sample by producing pro-inflammatory cytokines such as IL-6 during a stimulation period. The cell culture supernatant containing the released IL-6 cytokines is then analyzed with the PeliKine compact human IL-6 ELISA assay kit.

Regulatory requirements as outlined for example, by the European Pharmacopeia (Chapter 2.6.30) describe the following options for routine use of the MAT assay: the quantitative test (Method A), the semi-quantitative test (Method B), and the reference lot comparison test (Method C). This quick start guide is describing an example employing Method A or B. The "historical LOD" for the PyroCell™ MAT assay system is 0.02 EU/mL. For more details of the test procedure please refer to the PyroCell™ MAT System User Guide for Routine Use.

Day 1: Stimulation of pMAT Cells

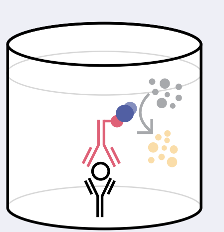
- Prepare endotoxin dilutions.
- Prepare test samples on the cell culture plate.
- Thaw pMAT Cells, transfer to the plate and incubate for 18-24h.

- Overnight coating of the ELISA plate.

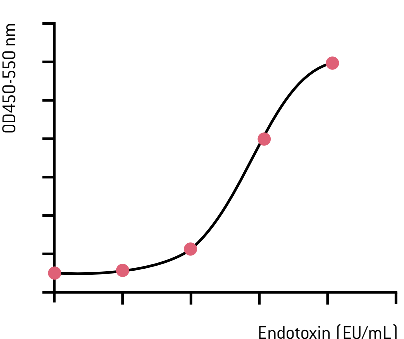


Day 2: IL-6 ELISA assay

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



Calculate the pyrogenicity of the test sample.



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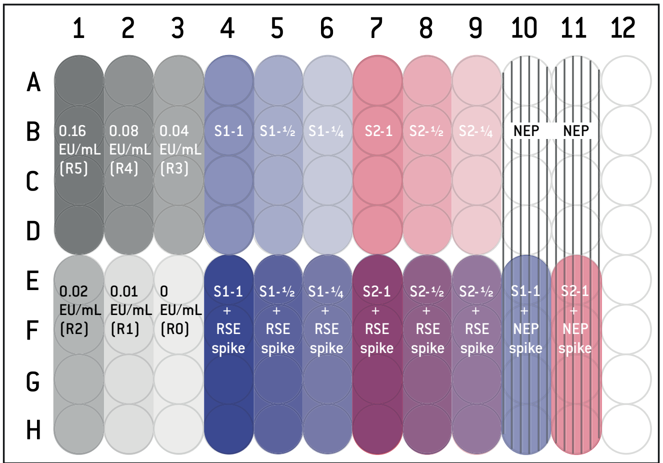
Day 1: Stimulation of pMAT Cells



Column 1-3: 5-point standard curve (0.16 – 0.08 – 0.04 – 0.02 – 0.01 EU/mL), and a blank sample.
Column 4-6 & 7-9: test samples (3 dilutions each) with and without control endotoxin spike (0.04 EU/mL).
Column 10-12: test sample or optional NEP control (spiked into complete medium and sample S1-1 and S2-1).

Solutions needed

- 1 mL reference standard endotoxin (RSE) dilution, 0.16 EU/mL (by serial dilution).
- 2 mL of endotoxin spike dilution, 0.08 EU/mL (3 samples).
- 2.5 mL of test sample dilutions (up to 3 samples).
- 1 mL non-endotoxin pyrogen (NEP) dilution (optional control, replace the 3rd sample).

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.
- The highest dilution on the plate shall not exceed the maximum valid dilution (MVD).
- Final dilution in the plate: 2-fold for all samples.

Endotoxin dilutions

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

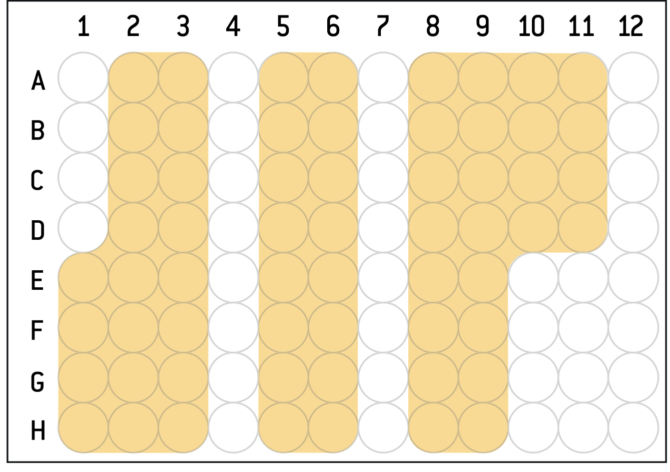
Step 1: Serial dilution

Step 2: Spike solution

2000 EU/mL	100 EU/mL	2 EU/mL	0.16 EU/mL	0.08 EU/mL
RSE stock solution in LAL water	50 µL RSE dilution, 950 µL IMDM	20 µL RSE dilution, 980 µL complete medium	80 µL RSE dilution, 920 µL complete medium	80 µL RSE solution 1920 µL complete medium

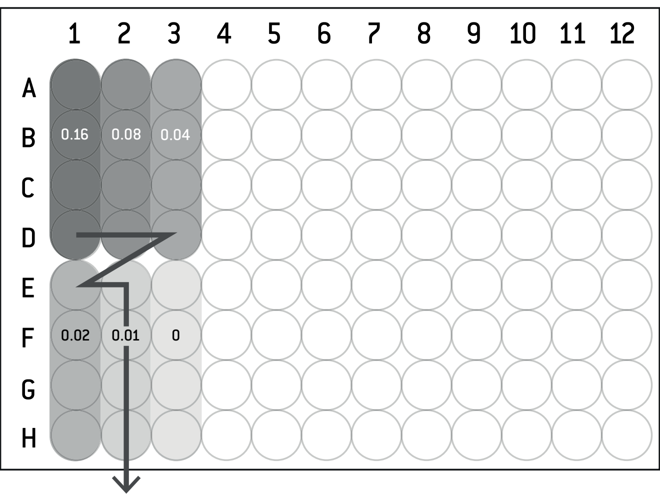
PyroCell™ MAT System quick start guide

Prepare the cell culture plate



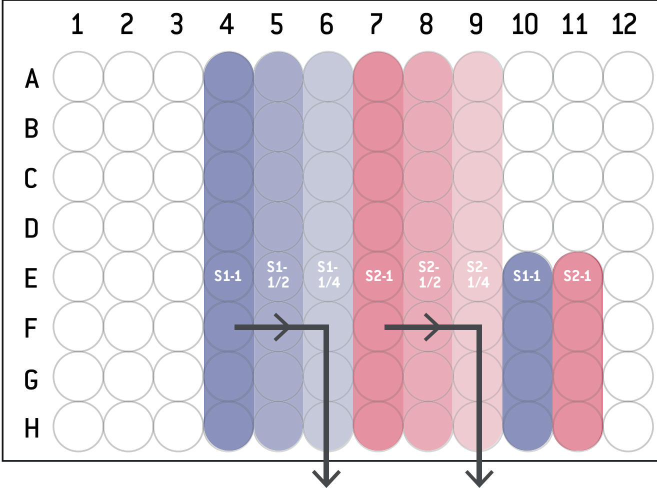
Step 3: Transfer complete medium

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



Step 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-H2 as illustrated.
- Discard 100 µL from E2-H2.



Step 5: Prepare the sample dilution

- 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.

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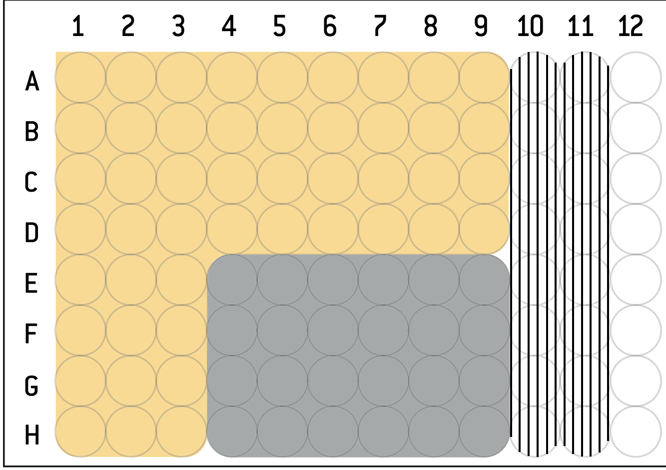
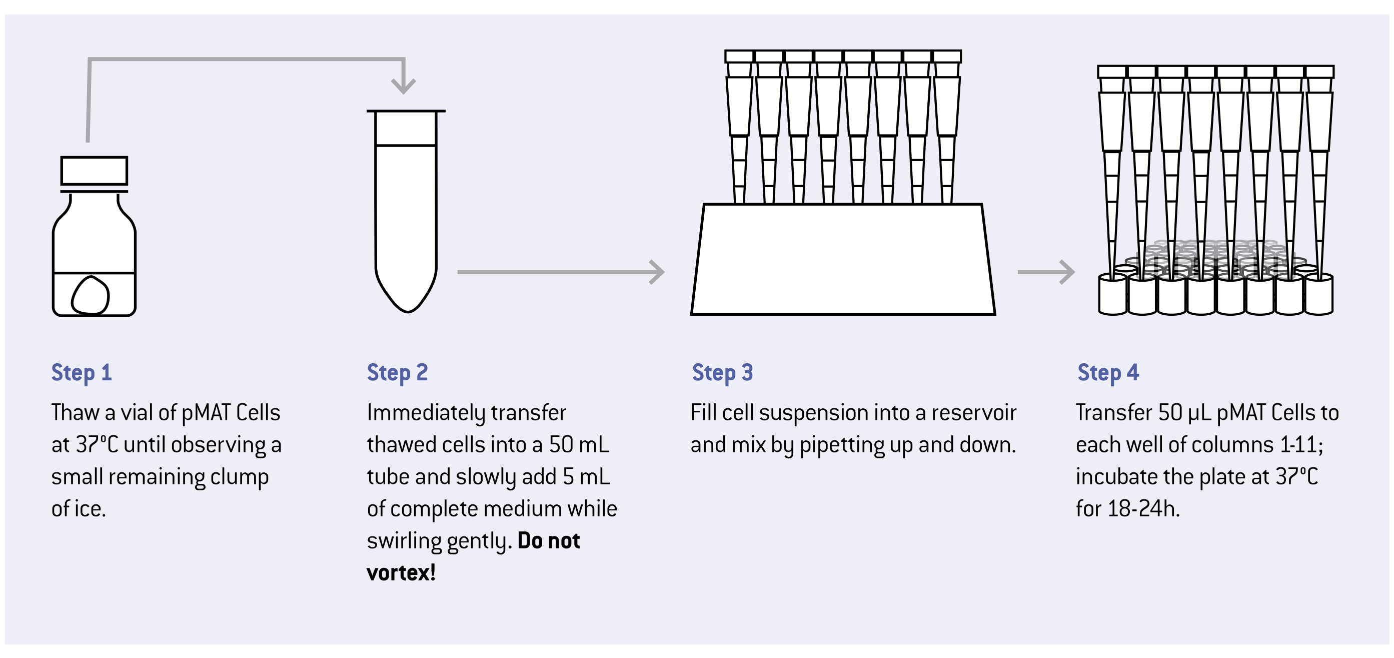


Step 6: Add endotoxin spike and optional, NEP control

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

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 and mix by pipetting up and down.

Step 4

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

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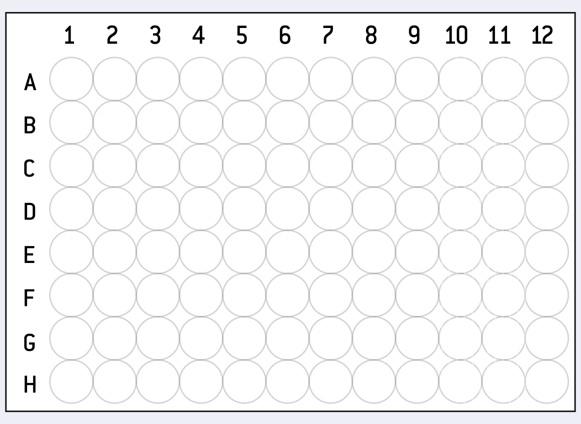
Day 2: IL-6 ELISA assay

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).

Step1: Overnight coating of the ELISA plate (day 1)

- Dissolve content of one coating buffer capsule in 100 mL distilled water, incubate 5 minutes.
- Mix 12 mL of coating buffer with 120 µL coating antibody.
- Add 100 µL coating buffer to each wells of the microtiter plate and incubate over night at room temperature.

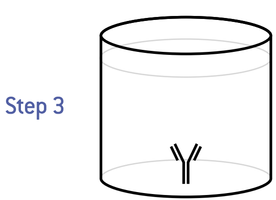


Step 2

Harvesting the cell culture supernatants

- Transfer supernatants from the cell culture plate (day1) into a fresh plate (plate 1).
- Dissolve 15 mL 5-fold concentrated HPE buffer in 60 mL distilled water.
- Prepare a dilution of each supernatant (e.g. a 1:5 dilution) by adding 120 µL of 5-fold concentrated HPE buffer into each well of a fresh plate (plate 2). Transfer 30 µL supernatant from plate 1 to the corresponding well of plate 2. Mix by pipetting up and down.

Step 3

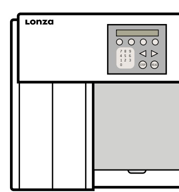


ELISA step	Wash		Blocking of unspecific binding sites
Reagent	PBS Buffer (1 PBS tablet in 200 mL distilled water)	→	Blocking Buffer (500 µL Blocking Reagent in 25 mL PBS)
Use	300 µL/ well 4 times		200 µL/ well 1h incubation at 18-25°C

PyroCell™ MAT System quick start guide

Step				
Step 4	ELISA step	Wash	→	Binding of Cytokines from supernatant (Optional, add IL-6 Standard Dilutions)
	Reagent	Wash Buffer (50 mL concentrate in 950 mL distilled water)		Supernatant dilutions (plate 2)
	Process	300 µL/ well 5 times		100 µL/ well 1h incubation at 18-25°C
Step 5	ELISA step	Wash	→	Binding of anti-cytokine antibody
	Reagent	Wash Buffer (from Step 3)		Biotinylated anti-IL-6 antibody (120µL antibody + 12 mL HPE)
	Process	300 µL/ well 5 times		100 µL/ well 1h incubation at 18-25°C
Step 6	ELISA step	Wash	→	Binding of HRP conjugate
	Reagent	Wash Buffer (from Step 3)		Streptavidin-HRP onjugated antibody (1.2 µL antibody + 12 mL HPE)
	Process	300 µL/ well 5 times		100 µL/ well 30 min incubation at 18-25°C
Step 7	ELISA step	Wash	→	Color reaction
	Reagent	Wash Buffer (from step 3)		TMB substrate, 100 µL
	Process	300 µL/ well 5 times		Up to 10 min incubation (dark) at 18-25°C Add Stop Solution, 100 µL

Step 8



Read results

- Determine the absorptions (OD) at 450 nm and 540-590 nm in a plate reader (e.g. ELx808 Reader) within 30 minutes.
- Analyze results.

MAT reagents and pyrogen-free materials

Product name	Cat. No.
PyroCell™ MAT System, IL-6	00254714
PyroCell™ MAT System, IL-1 beta	00254715
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	00190039
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
ELx808™ Reader with filters (450 nm & 540-590 nm)	25-315S
(optional) Non-endotoxin pyrogens	

What you also need

- 50 mL tubes (cell-culture grade, endotoxin-free)
- Bottles and test tubes (buffer preparation)
- Volumetric & 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 Documents

- Certificate of Analysis (CoA), www.lonza.com/coa
- PyroCell™ MAT System User Guide for Routine Use
- PyroCell™ MAT Analytics Guide

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