Instructions for use

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MAT Cell Set

REF M2016

Σ 288

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For research use only

General information

Pharmaceutical products intended for parenteral use must be free of pyrogens (fever–inducing substances), which can originate from bacteria, viruses and fungi. The monocyte activation test (MAT) is intended as an in vitro-based assay which determines pyrogenic activity in pharmaceutical products by measuring cytokine production of human monocytes. The MAT detects endotoxin and non-endotoxin pyrogens that are relevant to humans. The European Pharmacopoeia (Ph. Eur. chapter 2.6.30) is the main guideline regarding MAT procedure and product-specific validation[1].

Principle of the test

The MAT measures a response of the human innate immune system. Monocytes, the key cells of innate immunity, respond to the presence of pyrogens by producing a number of cytokines, which can be measured by Enzyme-Linked Immunosorbent Assays (ELISA). The pooled MAT (pMAT) Cells consist of a pool of cryo-preserved peripheral blood mononuclear cells (PBMC) isolated from 4 healthy human donors. The pMAT Cells are thawed and incubated with the test sample. Following incubation at 37°C, the cell culture supernatant is collected and analysed for the presence of the cytokine interleukin-6 (IL-6) by ELISA[2].

Package contents

pMAT Cells	3x1 mL	green caps	
MAT Culture Medium Supplement	3x2.1 mL	yellow caps	REF M201602

- The vial of pMAT Cells is labelled with two codes;
    - The first code (68-XXXX) refers to the lot org (original lot) number which can also be found on the label of the box and the Certificate of Analysis
    - The second code (P-XXX) refers to the individual vial code
- After opening, all reagents must be used immediately and cannot be re-frozen
- Consult the Certificate of Analysis for additional information

Additional materials and/or equipment

The following equipment and materials are required but not provided:
- Freezer (-80°C or lower)
- Laminar airflow cabinet
- CO_2-incubator at 37°C with 5% CO_2
- Water bath at 37°C
- Vortex mixer
- Microplate photometer for measuring absorbance at 450 nm, with a reference wavelength set between 540 and 590 nm
- Adjustable multichannel pipettor (30 - 300 μL)
- Adjustable pipettes (5 - 1000 μL)
- Appropriate sterile, non-pyrogenic pipette tips
- Sterile, non-pyrogenic polystyrene disposable pipettes
- Sterile, non-pyrogenic borosilicate glass tubes
- Sterile, non-pyrogenic 96-well flat bottom microplates suitable for cell culture
- Non-pyrogenic pipetting reservoir
- 96-well round bottom, untreated microplate for harvesting supernatant from cell culture
- Sterile, non-pyrogenic 50 mL conical polypropylene centrifuge tubes
- Iscove's Modified Dulbecco's Media (IMDM; 40 mL from a new, unopened bottle) from Lonza (REF: BE12-722F)
- Endotoxin reference standard that has been calibrated against the International Standard, e.g. endotoxin standard Biological Reference Preparation (BRP) from the European Pharmacopoeia
- Sterile, non-pyrogenic distilled or deionised water to reconstitute endotoxin standard
- Human IL-6 ELISA, e.g.:
    - PeliKine Human IL-6 ELISA Rapid Set A+B (REF M2018+M2019)
- Ph. Eur. chapter 2.6.30

Optional
- Repeating pipette
- Automated microplate washer
- Microplate shaker

Precautions

- Not suitable for in vitro diagnostic use. The MAT Cell Set is not intended for the detection of pyrogens in clinical samples or as an aid in the diagnosis of human disease.
- All MAT Cell Set reagents must be stored at -80°C or lower in the original packaging. Check your freezer for appropriate alarm settings.
- Thawed reagents must be used immediately and cannot be refrozen.
- Leaking or damaged vials cannot be used.
- Reagents (unopened or opened) cannot be used beyond the expiration date, which is printed on the label of the box.
- Do not use reagents with any evidence of turbidity or microbial contamination.
- Components of MAT Cell Set contain foetal bovine serum.
- The user must be trained and familiar with sterile and non-pyrogenic cell handling and with ELISA test procedures. The test shall be carried out by authorised and well-trained laboratory personnel only.
- Although the components of MAT Cell Set are tested for infectious diseases and found negative (see Certificate of Analysis for further details), the reagents cannot be assumed to be free from infectious agents and must be handled with appropriate care
- Care must be taken in the use and disposal of each container and its contents. Waste disposal, after completion of the test, must be performed according to your laboratory regulations.
- To prevent errors it is strongly recommended to have all required calculations reviewed by a second person.

Abbreviations

BRP	Biological Reference Preparation
CLC	Contaminant Limit Concentration
ELISA	Enzyme-Linked Immunosorbent Assay
Ph. Eur.	European Pharmacopoeia
EE/mL	Endotoxin Equivalents per millilitre
EU/mL	Endotoxin Units per millilitre
IL-6	Interleukin-6
IMDM	Iscove's Modified Dulbecco's Media
IPA	Isopropyl Alcohol
MAT	Monocyte Activation Test
MVD	Maximum Valid Dilution
OD	Optical density
PBMC	Peripheral Blood Mononuclear Cell

Definitions

The MAT is used to demonstrate that the amount of pyrogens in the test product does not exceed the Contaminant Limit Concentration (CLC). Therefore it is necessary to calculate the Maximum Valid Dilution (MVD) based on the test sensitivity.

1.1 Calculation of the Maximum Valid Dilution (MVD) and Contaminant Limit Concentration (CLC)

The Maximum Valid Dilution (MVD) is defined as the maximum allowable dilution of a sample at which the contaminant limit can be determined.[1] The MVD is calculated using the following expression:

$$\text{MVD} = \frac{\text{CLC x C}}{\text{Test sensitivity}}$$

CLC = Contaminant Limit Concentration
C = Concentration of test solution
Test sensitivity = The lowest endotoxin reference standard concentration on the standard curve whose response exceeds the cut-off value.

The CLC is calculated using the following expression:

$$\text{CLC} = \frac{K}{M}$$

K = threshold pyrogenic dose of endotoxin per kilogram of body mass
M = maximum recommended bolus dose of product per kilogram of body mass

1.2 Calculation of the cut-off value

The test sensitivity is the lowest concentration on the endotoxin standard curve. For each test it is to be confirmed that the test sensitivity response exceeds the cut-off value. Test sensitivity is determined using the endotoxin standard curve and is expressed as endotoxin equivalents per millilitre (EE/mL). The cut-off value is expressed in OD and is calculated using the following expression[1]:

$$\text{cut-off value} = \bar{x} + (3s)$$

$\bar{x}$ = mean of the responses obtained for the 4 blank replicates
s = standard deviation of the responses obtained for the 4 blank replicates

Test sensitivity of the MAT Cell Set is 0.02 EE/mL, in combination with the PeliKine Human IL 6 ELISA Rapid Set. Consult the Certificate of Analysis for additional information. The test sensitivity should be confirmed (> cut-off value) in each test to confirm the MVD calculation.[1]

NOTE: Endotoxin concentrations stated throughout this Instructions For Use are given as **concentrations per sample** (i.e. prior to addition of additional (spiked) media and pMAT Cells to the samples). The final concentration in the reaction well is half of the stated concentrations: The total reaction volume in a single well is 200 μL, which consists of 100 μL sample, 50 μL of media or spiked media

and 50 µL of cells.

Test procedure

The MAT consists of two main steps:

1) the MAT cell incubation with the standards and test samples
2) the IL-6 ELISA

During step 1, all equipment and materials coming into contact with the culture components need to be non-pyrogenic. Work in a laminar airflow cabinet, wear gloves, and clean the exterior of equipment and materials with 70% Isopropyl Alcohol (IPA) prior to the experiment. Step 2 (ELISA) can be performed on a regular lab bench (no requirement to work in a laminar airflow cabinet).

Ph. Eur. chapter 2.6.30 describes two test methods: the semi-quantitative test (Method 1) and the reference lot comparison test (Method 2). Prior to selection of one of these methods for routine use, preparatory testing (also known as product-specific validation) needs to be performed to ensure that the product will not interfere with the assay (i.e. that endotoxin and non-endotoxin pyrogens can be detected using the chosen method and that the product does not interfere in IL-6 ELISA). The MAT Cell Set may be used for both test methods stated in Ph. Eur. 2.6.30. This IFU describes the procedure for Method 1, refer to Ph. Eur. 2.6.30 for details.

Preparation of complete medium

The complete medium used for the MAT cell incubation consists of IMDM supplemented with the provided MAT Culture Medium Supplement (M201602). Always use an unopened bottle of IMDM and prepare complete medium on the day of the experiment.

1. Thaw MAT Culture Medium Supplement in a water bath at 37°C and add the entire contents (2.1 mL) to a sterile, non-pyrogenic 50 mL tube containing 33 mL IMDM and mix gently by inversion of the closed tube

IMPORTANT: a minimum of 1 mL IMDM **without** addition of the culture medium supplement mix is needed to make the first dilution of the endotoxin standard. Preparing the first dilution with complete medium will result in a non-accurate endotoxin standard curve.

2. Allow the complete medium to reach room temperature (18 - 25°C) and mix gently before use. Use complete medium within eight hours.

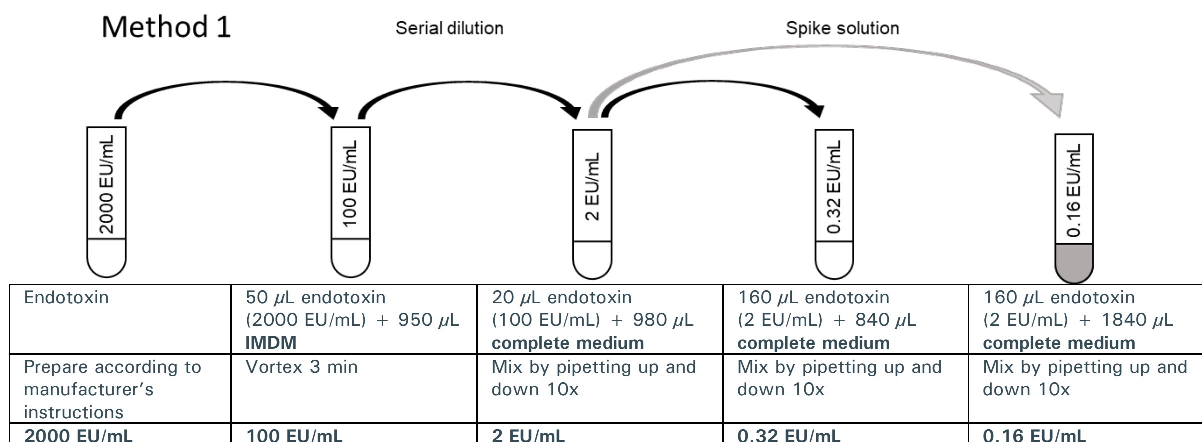
Preparation of the endotoxin standard curve

The endotoxin (not included) standard curve is used to determine the (back-calculated) cut-off and to estimate the amount of pyrogen in the test sample. Include an endotoxin standard in each microplate as the OD values of the endotoxin standard dilutions and samples may vary from plate to plate. Chapter 2.6.30 of the European Pharmacopoeia requires a standard curve that consists of at least 4 replicates of 6 different endotoxin concentrations and a blank for a non-linear fit. We recommend to use 6 different endotoxin concentrations starting from 0.32 EU/mL with twofold dilutions down to 0.01 EU/mL and a blank. A suggested plate layout is given in figure 1.

row ID	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	0.32 EU/mL	0.16 EU/mL	0.08 EU/mL	0.04 EU/mL								
C	(R6)	(R5)	(R4)	(R3)	S1/f	S1/f1	S1/f2	S2/f	S2/f1	S2/f2		
D												
E												
F	0.02 EU/mL	0.01 EU/mL	0 EU/mL		S1/f +	S1/f1 +	S1-f2 +	S2/f +	S2/f1 +	S2/f2 +		
G	(R2)	(R1)	(R0)		spike	spike	spike	spike	spike	spike		
H												

Figure 1: Suggested plate layout and pipetting scheme for method 1. Wells in shades of grey: Endotoxin standard concentrations R0 – R6 in EU/mL. Wells in shades of yellow and green: test samples S1 and S2 in 3 dilutions (f, f1 and f2). Dotted wells indicate test samples spiked with an endotoxin concentration near the middle of the endotoxin standard curve (0.08 EU/mL). Wells E4-H4 and columns 11 and 12 remain empty in this suggested plate layout.

1. Reconstitute the endotoxin standard to 2000 Endotoxin Unit (EU)/mL following the manufacturer's instructions (see Figure 2). **Prepare the endotoxin standard curve on the day of the experiment.**
2. Add 950 µL **IMDM** (WITHOUT MAT CULTURE MEDIUM SUPPLEMENT) to a sterile, non-pyrogenic borosilicate glass tube and to this add 50 µL of 2000 EU/mL endotoxin to reach a concentration of 100 EU/mL (see Figure 2). Vortex thoroughly for 3 minutes.
3. Add 980 µL **complete medium** to a sterile, non-pyrogenic borosilicate glass tube and to this add 20 µL of 100 EU/mL endotoxin to reach a final concentration of 2 EU/mL (see Figure 2). Mix thoroughly by pipetting up and down for 10 times, with a volume of at least 500 µL.
   NOTE: Do not vortex and prevent the formation of air bubbles as this will result in decreased cell reactivity towards endotoxin.
4. Add 840 µL **complete medium** to a sterile, non-pyrogenic borosilicate glass tube and to this add 160 µL of 2 EU/mL endotoxin to reach a final concentration of 0.32 EU/mL (see Figure 2). Mix thoroughly by pipetting up and down for 10 times, with a volume of at least 500 µL.
   NOTE: Do not vortex and prevent the formation of air bubbles as this will result in decreased cell reactivity towards endotoxin.



Endotoxin	50 μL endotoxin (2000 EU/mL) + 950 μL **IMDM**	20 μL endotoxin (100 EU/mL) + 980 μL **complete medium**	160 μL endotoxin (2 EU/mL) + 840 μL **complete medium**	160 μL endotoxin (2 EU/mL) + 1840 μL **complete medium**
Prepare according to manufacturer's instructions	Vortex 3 min	Mix by pipetting up and down 10x	Mix by pipetting up and down 10x	Mix by pipetting up and down 10x
2000 EU/mL	**100 EU/mL**	**2 EU/mL**	**0.32 EU/mL**	**0.16 EU/mL**

Figure 2: Schematic overview of endotoxin dilutions for Method 1

5. Add 200 μL of 0.32 EU/mL endotoxin to well A1 to D1 (see Figure 1).
6. Add 100 μL of **complete medium** to the other wells used for the standard curve.
7. Make a twofold dilution by pipetting 100 μL from R6 (0.32 EU/mL) to R5 (0.16 EU/mL) and mix thoroughly by pipetting up and down for 10 times. Prevent the formation of air bubbles.
8. Repeat this until R1 (0.01 EU/mL) and discard the final 100 μL.
9. Save the remainder of the 2 EU/mL endotoxin solution prepared at step 3 for preparation of endotoxin spiked samples (step 7 of chapter "Preparation of the test samples").

Preparation of the test samples

Ph. Eur. chapter 2.6.30 describes that all test samples are run in 4 replicates. An example of a plate layout is given in figure 1.

A maximum of 2 products can be tested on a single plate (see also figure 1). **Prepare the test samples on the day of the experiment.** (NOTE: the procedure below describes a two-fold sample dilution and only serves as an example. The sample dilutions should be chosen after evaluation of the data from the product-specific validation).

1. Add 200 μL of sample (S1/f) to each of the 8 wells as indicated in Figure1.
2. Add 100 μL of **complete medium** to the other wells used for the sample dilutions.
3. Make a two-fold dilution by pipetting 100 μL from S1/f to S1/f1 and mix thoroughly by pipetting up and down for 10 times. Prevent the formation of air bubbles.
4. Repeat this until S1/f2 and discard the final 100 μL.
5. If a second sample is to be tested: repeat step 1 to 4 for sample S2.
6. Add 50 μL of **complete medium** to the standards and samples without endotoxin spike.
7. Add 1840 μL **complete medium** to a sterile, non-pyrogenic borosilicate glass tube and to this add 160 μL of 2 EU/mL endotoxin to reach a final concentration of 0.16 EU/mL (see Figure 2). Mix thoroughly by pipetting up and down for 10 times, with a volume of at least 1 mL. Do not vortex and prevent the formation of air bubbles.
8. Add 50 μL of 0.16 EU/mL endotoxin to the spiked samples (+ spike). The endotoxin spike concentration in the sample corresponds to the 0.08 EU/mL value from the endotoxin standard curve (see also the note in chapter **Definitions**).

MAT Cell incubation

1. Take the vial of pMAT Cells from the -80°C freezer and **immediately thaw it in a water bath at 37°C until a small clump of ice remains (<5 minutes).**
2. Transfer the entire contents of the vial (1 mL) into a 50 mL tube and immediately but slowly add 5 mL complete medium (at room temperature) while gently swirling the tube (<5 minutes). Do not vortex or vigorously pipet, take care not to form air bubbles.
3. Transfer mixture to a non-pyrogenic pipetting reservoir. Immediately add 50 μL of the pMAT Cells to the appropriate wells
4. Incubate the microplate with lid in a CO_2-incubator at 37°C with 5% CO_2 for 18-24 hours.
5. Harvest 100-150 μL of supernatant by carefully placing the pipette tip at the left or right bottom side of the well and transfer this to a new 96-well round bottom, untreated microplate.
6. Measure IL-6 production by ELISA. Alternatively, the supernatant may be frozen at ≤-18°C . Storage time and the temperature of the frozen supernatant may be product-dependent and needs to be determined by the user.

IL-6 ELISA procedure

The pMAT Cells have been validated using the PeliKine Human IL-6 ELISA Rapid Set A (REF M2018) and PeliKine Human IL-6 ELISA Rapid Set B (REF M2019) (not included). The test sensitivity is 0.02 EE/mL. Consult the Certificate of Analysis for additional information. Follow the manufacturer's instructions for use of the ELISA kit. In our experience using the PeliKine Human IL-6 ELISA Rapid Set, a sample dilution of 5 fold gives the optimal result when using the recommended endotoxin standard curve. Please establish your own optimal dilution. An IL-6 standard curve may be included in the ELISA as a quality control, but is not necessary to determine the pyrogenic activity of the sample. Refer to the original IFU of the PeliKine Human IL-6 ELISA Rapid Set A (REF M2018) and PeliKine Human IL-6 ELISA Rapid Set B (REF M2019) for information on principle of the test, storage and stability, contents of the kit, precautions for use, and additional information.

Components marked with "[(a)]" are part of the PeliKine Human IL-6 ELISA Rapid Set A (ref. no. M2018), components marked with "[(b)]" are part of the PeliKine Human IL-6 ELISA Rapid Set B (ref. no. M2019).

General guidelines

1. **Allow all reagents to calibrate to room temperature (18-25°C) prior to use**, with the exception of the streptavidin-HRP conjugate which has to be kept at -32°C to -18°C to ensure stability.
2. The complete assay must be performed at room temperature (18-25°C) without shaking. Shaking is optional, step 5 (incubation of supernatant and biotinylated antibody) and step 8 (incubation of streptavidin-HRP conjugate) of section 'Procedure' can be performed on a horizontal plate shaker (700 ± 100 rpm).
3. The stated volumes are for *one* ELISA plate.
4. If more than one plate is assayed, first complete the steps of adding supernatant and biotinylated antibody (as described in the second and third points of section "procedure" step 4) for the first plate, then repeat and complete these steps for each plate.
5. Mix all reagents thoroughly but gently before use (without foaming).
6. Centrifuge all vials before use (1 minute 3000 x g).
7. Do not allow wells to stand uncovered or dry for extended periods between incubation steps.
8. Carefully remove all air bubbles from the wells before incubation.
9. The opalescent working-strength HPE-dilution buffer can be stored for up to one week at 2-8°C.
10. The working-strength wash buffer can be stored up to 2 months at 2-8°C.
11. Protect TMB substrate solution from prolonged exposure to light.
12. The procedure in section "procedure" is designed for a 5x supernatant dilution, for examples of preparation of other dilutions refer to section "Examples of supernatant dilutions".

Procedure

1. Prepare working-strength HPE-dilution buffer:
   - Add 15 mL 5-fold concentrated HPE-dilution buffer[a] to 60 mL distilled water.
2. Prepare IL-6 standard in working-strength HPE-dilution buffer: (Optional: An IL-6 standard curve may be included in the ELISA as a quality control, but is not necessary to determine the pyrogenic activity of the sample.)
   - Label 7 tubes, one tube for each dilution: 450, 150, 50, 16.7, 5.6, 1.9, 0.6 pg/mL(final concentration in the ELISA plate).
   - Pipette 49 µL of working-strength dilution buffer into the tube labelled 450 pg/mL and 80 µL of working-strength dilution buffer into the other tubes.
   - Transfer 63 µL of the IL-6 standard[a] (4000 pg/mL) into the first tube labelled 450 pg/mL, mix well and transfer 40 µL of this dilution into the second tube labelled 150 pg/mL.
   - Repeat the serial dilutions five more times by adding 40 µL of the previous tube of diluted standard to the next tube containing 80 µL of dilution buffer.
   - It is recommended to prepare two separate series (duplicate) for each assay.
3. Prepare the working-strength biotinylated antibody (81x diluted, final dilution in the ELISA plate is 101x):
   - Add 120 µL biotinylated antibody[a] to 9.6 mL HPE buffer. Mix by inverting the tube 10 times.
   - Add this mix to a reagents reservoir.
4. Add the supernatant and the working-strength biotinylated antibody to the IL-6 ELISA pre-coated plate:
   - Homogenise the harvested supernatant by pipetting up and down for 5 times and add 20 µL to the corresponding wells in the IL-6 ELISA pre-coated plate.
   - Add 20 µL of the prepared IL-6 standard to columns 11 and 12 of the pre-coated plate and 20 µL working-strength HPE-dilution buffer to the blank wells (H11 and H12).
   - Immediately add 80 µL working-strength biotinylated antibody to the wells, containing supernatant and standard curve, with a multichannel pipette and mix 3x gently by pipetting up and down; change the pipetting tips each row.
   - Cover the pre-coated plate with a seal and **incubate for 1 hour at 18-25°C.**
5. Prepare wash buffer:
   - Prepare working-strength wash buffer by adding 50 mL of the wash buffer concentrate[b] (total content of the bottle) to 950 mL distilled water.
6. Just before the washing step, prepare the working-strength streptavidin-HRP conjugate:
   - Add 2 µL 10,000-fold concentrated streptavidin-poly-HRP conjugate[a] to 20 mL working-strength HPE-dilution buffer. Mix by inverting the tube 10 times.
7. After 1 hour of incubation wash the plate 5 times with wash buffer (volume > 300 µL) manually or on a plate washer. After the final emptying the plate should be dry.
8. Add 100 µL of working-strength streptavidin-HRP conjugate to the appropriate wells and cover the microplate with adhesive seal. Gently agitate the microplate by tapping the edge of the plate for a few seconds to mix contents of each well and **incubate for 30 minutes at 18-25°C**.
9. After 30 minutes of incubation wash the plate 5 times with wash buffer (volume > 300 µL) manually or on a plate washer. After the final emptying the plate should be dry.
10. Add 100 µL TMB substrate solution[b] to all wells. Gently agitate the microplate by tapping the edge of the plate for a few seconds to mix contents of each well and **incubate for 10 minutes (recommended) at 18-25°C in the dark** (do not use aluminium foil, but place the microplate e.g. in a cupboard).
11. After 10 minutes of incubation add 100 µL of stop solution[b] to all wells. **After stopping the substrate reaction the colour is stable for maximally 30 minutes.**
12. Place the microplate in an ELISA reader and record absorbance at 450 nm, with a reference wavelength set between 540 and 590 nm (subtract the reference wavelength measurement from the measurement at 450 nm if the software does not subtract this automatically).

Examples of supernatant dilutions

If a supernatant dilution other than 5x is preferred, then pre-dilute the supernatant (in e.g. a U bottom plate) according to the examples in the table below. Mix the supernatant, together with the working-strength HPE-dilution buffer, 5x and then transfer 20 µL pre-diluted supernatant to the IL-6 ELISA pre-coated plate.

Dilution	Supernatant	1x HPE buffer
10x	30 µL	30 µL
20x		90 µL
50x		270 µL

Results

Every in-house or on-line available software method for calculation of endotoxin concentrations may be used. A general method for calculation is given below. Refer to Ph. Eur. 2.6.30 for a detailed description.

1. For each quadruplicate measurement, calculate the net absorbance values by subtracting the reference wavelength set between 540 and 590 nm from the OD450 nm reading.
2. (optional) Remove outliers caused by documented deviations to work instructions, e.g. pipetting errors.
3. (optional) Apply a sound statistical method to identify outliers within each group of replicates (e.g. Dixon method with 95% Q-critical value).
4. Calculate the average OD value for each of the replicate measurements.
5. Plot the OD values on the y-axis and the endotoxin standard concentrations on the x-axis and perform a regression. A five-parameter logistic non-linear regression model is recommended.
6. With the regression equation calculate the concentration of each product dilution. Alternatively, locate the average net absorbance value for each product dilution on the y-axis and follow a horizontal line intersecting the regression line. At the intersection read the endotoxin concentration from the x-axis on a vertical line.
7. Multiply the obtained endotoxin concentration with the dilution factor of the product sample to obtain the contaminant concentration in the sample.
8. Evaluate that the cut-off < test sensitivity (see chapter **Definitions**).

A typical endotoxin standard curve using the PeliKine Human IL-6 ELISA Rapid Set is shown in Figure 3. This standard curve was generated for demonstration purposes only. A pyrogen standard curve must be run with each plate.

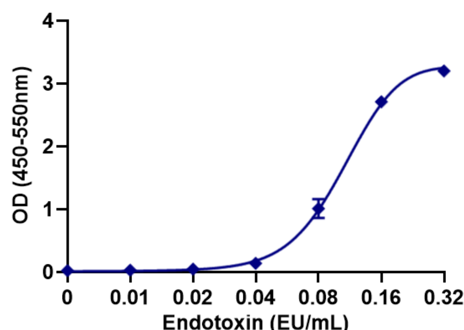


Figure 3: Example of an endotoxin standard curve

According to Ph. Eur. 2.6.30, the standard curve is valid when the following two acceptance criteria are met[1]:

1. There should be a good fit between the data points and the chosen regression model. This can be evaluated visually (e.g. by assessment of regression and/or residual plots) or for a non-linear regression model with a lack-of fit test ($p > 0.05$).
2. The coefficient of determination should be above 0.975.

Interpretation

Refer to Ph. Eur. 2.6.30 for a detailed description.

Specifications

The values cited for specific performance characteristics of the test represent typical results obtained using the PeliKine Human IL-6 ELISA Rapid Set A (REF M2018) and PeliKine Human IL-6 ELISA Rapid Set B (REF M2019). Consult the Certificate of Analysis for additional information.

Performance characteristics endotoxin

Consult the Certificate of Analysis on performance characteristics of endotoxin.

Performance characteristics of tested non-endotoxin pyrogens:

Consult the Certificate of Analysis on performance characteristics of non-endotoxin pyrogens.

Limitations

- MAT Cell Set has been designed and validated using IL-6 as read-out for pyrogenic activity. Other cytokines may be suitable as read-out, but have to be validated by the user.
- A reference sample with a known concentration of pyrogen (not included) may be used for quality control purposes.

- pMAT Cells are reactive towards the non-endotoxin pyrogens listed in the Certificate of Analysis. pMAT Cells may be responsive to other non-endotoxin pyrogens, but these have to be tested by the user.

References

1. European Directorate for the Quality of Medicines. *Ph. Eur.* chapter 2.6.30 (07/2024): Monocyte Activation Test.
2. Solati S, Aarden L, Zeerleder S, Wouters D; An improved monocyte activation test using cryopreserved pooled human mononuclear cells. *Innate Immunity*. 2015 Oct;21(7):677-84.

Disclaimer

Sanquin Reagents products are guaranteed to perform as described in the original manufacturer's instructions for use. Sanquin Reagents declines all responsibility arising from any deviation thereof. The user is responsible for proper calculations and subsequent interpretations of the results. Sanquin Reagents is not liable for any claims, including third party claims, due to or caused by pyrogenic activity in pharmaceutical products. Consult the relevant chapters in the European Pharmacopoeia (Ph. Eur. 2.6.30) as well as other documents relevant for your specific purpose for utilization of the test.