Instructions for use

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PeliKine Human IL-6 ELISA Rapid Set B

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For research use only

General information

The PeliKine Human IL-6 ELISA Rapid Set is an enzyme-linked immunoassay (ELISA) for fast, reproducible and specific quantitative determination of IL-6 in MAT supernatant. This ELISA is validated in combination with Sanquin's monocyte activation test (MAT) kits: MAT Cell Set (M2016) and MAT Cell Set HS (M2017). The MAT is an in vitro-based assay which determines pyrogenic activity in pharmaceutical products by measuring cytokine production of human monocytes. Pharmaceutical products, intended for parenteral use, must be free of pyrogens (fever–inducing substances), which can originate from bacteria, viruses and fungi. The MAT detects endotoxin and non-endotoxin pyrogens that are relevant to humans. The European Pharmacopoeia (Ph. Eur. chapter 2.6.30) considers the MAT to be suitable as a replacement for the rabbit pyrogen test, after a product-specific validation[1]. Note that the PeliKine Human IL-6 ELISA Rapid Set consists of 2 kit parts: PeliKine Human IL-6 ELISA Rapid Set A (ref. no. M2018) and PeliKine Human IL-6 ELISA Rapid Set B (ref. no. M2019). The contents of this IFU (except for sections "Package contents" and "Precautions") are identical to the contents of the IFU for the PeliKine Human IL-6 ELISA Rapid Set A (M2018).

Principle of the test

The PeliKine Human IL-6 ELISA Rapid Set is a "sandwich-type" of enzyme immunoassay. This kit includes the proven antibodies (capturing [i.e. coating antibody] and biotinylated antibody) as found in the PeliKine Human IL-6 ELISA kit (M1916)[2]. Samples, containing IL-6 (i.e. MAT supernatant), together with a biotinylated anti-human IL-6 antibody, are added to the microtiter plate. The microtiter plates are pre-coated with anti-human IL-6 capturing antibody. In the microtiter plate, IL-6 is simultaneously captured to polystyrene microtiter wells and bound by the biotinylated anti-human IL-6 antibody. Non-bound material is then removed by washing. Subsequently, streptavidin-horseradish peroxidase (HRP)-conjugate is added, which binds to the biotinylated antibody. After removal of non-bound HRP conjugate by washing, substrate solution is added to the wells. A coloured product is formed in proportion to the amount of IL-6 present in the supernatant. After the reaction has been terminated by the addition of a stop solution, absorbance is measured in a microtiter plate reader.

Package contents

Name	Amount	Concentration	Appearance	Ref. no.
Human IL-6 ELISA pre-coated plates	3 x 96 wells	-	-	REF M191605
Wash buffer	2 x 50 mL	20-fold concentrated	White bottle	REF M180501
TMB substrate solution	1 x 40 mL	Ready for use	Brown bottle	REF M198004
Stop solution (0.18 M H_2SO_4)	1 x 40 mL	Ready for use	White bottle	REF M198005
Plate seals	12 x	-	-	-

- Store the PeliKine Human IL-6 ELISA Rapid Set B (ref. no. M2019) at 2-8 °C
- The flat-bottom microtiter plates are ready for use. All the wells are pre-coated with anti-human IL-6 capturing antibody. The microtiter plates are vacuum sealed in a plastic pouch containing desiccant. The microplates should be used immediately after opening and are one-time-use only.
- Consult the Certificate of Analysis for additional information.

Additional materials and/or equipment

The following equipment and materials are required but not provided:
- Microplate photometer for measuring absorbance at 450 nm, with a reference wavelength set between 540 and 590 nm
- Reagents reservoirs
- Distilled or deionised water
- Calibrated pipettes (5-1000 µL)
- Calibrated multichannel pipettes (30-300 µL)
- Beakers, flasks, cylinders, tubes and liquid containers necessary for preparation of reagents

Optional
- Automated microplate washer
- U bottom plate

Precautions

- Not suitable for in vitro diagnostic use. The PeliKine Human IL-6 ELISA Rapid Set is not intended for the detection of IL-6 in clinical samples or as an aid in the diagnosis of human disease.
- The PeliKine Human IL-6 ELISA Rapid Set B (ref. no. M2019) should be stored at 2-8°C.
- All kit components must be stored in the original packaging to allow for verification of the expiration date.
- Reagents (unopened or opened) cannot be used beyond the expiration date, which is printed on the label of each component.

- Leaking or damaged vials or bottles or other materials cannot be used.
- Only use the reagents supplied with the set, do not mix reagents from different kit lots.
- Reagents or remnants of reagents (e.g. dead volume) cannot be mixed with contents of freshly opened vials.
- Caps and vials/bottles are not interchangeable, caps must be replaced on the corresponding vials/bottles.
- Do not use reagents with any evidence of turbidity or microbial contamination.
- Reagents cannot be assumed to be free from infectious agents.
- Handle all samples with care to prevent transmission of blood-borne infections.
- Sodium azide inactivates HRP, do not use sodium azide-containing solutions, nor add sodium azide to the supplied materials.
- Wash buffer contains Merthiolate (0.001 % w/v).
- Do not add any preservative to the supplied reagents, they may have direct or indirect effects on the final colour development of the HRP-substrate system.
- Prior to the assay, frozen supernatant/samples should be thawed as quickly as possible in tap water (18-25°C), do not use 37°C or 56°C water baths for this purpose.
- Do not expose the TMB substrate solution to strong light during incubation or storage. The TMB substrate solution must be colourless when used; if the solution turns blue, it must be replaced.
- TMB substrate solution or stop solution should not be in contact with metals or metal ions, to avoid unwanted colour formation.
- Stop solution contains sulphuric acid, refer to the MSDS of M2019 for details.
- 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.
- For optimal performance of ELISA make sure that all pipets and systems are checked and under full maintenance service according to described procedures of the manufacturers.

Material safety data sheet

The Material safety data sheet for this product can be found on our website: www.sanquin.org/reagents by searching for the product number.

Abbreviations

ELISA	Enzyme-Linked Immunosorbent Assay
HPE	High Performance ELISA
HRP	Horseradish peroxidase
HS	Human serum
IL-6	Interleukin-6
LOD	Limit Of Detection
MAT	Monocyte Activation Test
Ph. Eur.	European Pharmacopoeia
TMB	3,3',5,5'-Tetramethylbenzidine

Test procedure

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 (80x diluted, final dilution in the ELISA plate is 100x):
   - 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 (see also Figure 1).
   - 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).

row ID	1	2	3	4	5	6	7	8	9	10	11	12
A	MAT supernatant										450 pg/mL IL-6	
B											150 pg/mL IL-6	
C											50 pg/mL IL-6	
D											16.7 pg/mL IL-6	
E											5.6 pg/mL IL-6	
F											1.9 pg/mL IL-6	
G											0.6 pg/mL IL-6	
H											Blank IL-6	

Figure 1: Suggested plate lay-out for the ELISA. Depicted concentrations of the IL-6 standard curve are final concentrations in the ELISA plate.

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

Consult Ph. Eur. chapter 2.6.30 and the IFUs for MAT Cell Set (M2016) and MAT Cell Set HS (M2017) for guidelines on data analysis.

Interpretation

Consult Ph. Eur. chapter 2.6.30 and the IFUs for MAT Cell Set (M2016) and MAT Cell Set HS (M2017) for guidelines interpretation of results.

Specifications

Consult the Certificate of Analysis on performance characteristics.

Limitations

- This ELISA kit has been designed for professional use only and for analysis of supernatant obtained from the MAT Cell Set (M2016) and MAT Cell Set HS (M2017). The user must be trained and familiar with pyrogen test and ELISA test procedures.
- Only the washing steps are validated by Sanquin on an automated microplate washer (Biotek 405 TS). The other steps, as described in this IFU, are validated by Sanquin with a manual testing procedure. All claims in this IFU are validated with the manual testing procedure (except for the washing steps). When using the kit on an ELISA automate, the test must be validated by the user before use. The claims in this IFU are not valid for the performance of this kit on an ELISA machine.
- The ELISA procedure is validated for a 5x MAT supernatant dilution. Other dilutions have to be validated by the user.
- This kit is validated with supernatant obtained from the MAT Cell Set (M2016) and MAT Cell Set HS (M2017). Unlike M1916, this kit is not validated for analysis of IL-6 in serum and plasma samples. The use of supernatant from other cell or supplement sources or other matrices has to be validated by the user.

References

1. European Directorate for the Quality of Medicines. *Ph. Eur.* chapter 2.6.30: Monocyte Activation Test.
2. Helle M, Boeije L, de Groot E, de Vos A, Aarden L; Sensitive ELISA for interleukin-6. Detection of IL-6 in biological fluids: synovial fluids and sera. *J.Immunol.Methods.* 1991 Apr 8;138(1):47-56.

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