

Instructions for Use

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PeliKine Human IL-6 ELISA MoAb Set A	REF M2021
PeliKine Human IL-6 ELISA MoAb Set B	REF M2022
347 version 01 Issued 21-Sep-2026 (en)	RUO <i>RUO-For Research Use Only. Not for use in diagnostic procedures.</i>

Intended Purpose

PeliKine Human IL-6 ELISA MoAb Set A,B (M2021, M2022) is a research use only device to be used by laboratory professionals to detect IL-6 in cell culture supernatant.

General Information

The PeliKine Human IL-6 ELISA MoAb is an enzyme-linked immunoassay (ELISA) for fast, reproducible and specific quantitative determination of IL-6 in cell culture supernatant. This ELISA was validated in combination with the Monocyte Activation Test (MAT) kits: MAT Cell Set (M2016) and MAT Cell Set HS (M2017). Note that the PeliKine Human IL-6 ELISA MoAb consists of 2 kit parts: PeliKine Human IL-6 ELISA MoAb Set A (M2021) and PeliKine Human IL-6 ELISA MoAb Set B (M2022).

Package Contents

- Leaflet 347-version_01
- M2021 and M2022 kit components are summarised below.

M2021 Kit Component	Cat. No.	Content	Active Ingredients/Description
IL-6 ELISA standard	M191603	1 x 750 µL	See label for concentration
Anti-IL-6 biotinylated MoAb, 101 x	M202104	1 x 375 µL	Biotinylated monoclonal anti-IL-6 antibody
Streptavidin-poly-HRP, 10000x	M1942	1 x 20 µL	Horseradish peroxidase coupled to streptavidin
HPE buffer, 5x	M1940	1 x 55 mL	5% Sheep Serum

M2022 Kit Component	Cat. No.	Content	Active Ingredients/Description
IL-6 ELISA MoAb pre-coated plates	M202202	3 x 96 wells	Pre-coated microtiter plate (anti-IL-6), for 3 ELISA runs
Wash buffer, 20x	M1805	2 x 50 mL	Sodium phosphate based
TMB substrate solution	M198004	1 x 40 mL	100% TMB
Stop solution	M198005	1 x 40 mL	0.18M H2SO4
Plate seals	n.a.	12 pieces	Adhesive seals

Consult the Certificate of Analysis for additional information.

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

Materials Required (not provided)

- Microplate photometer for measuring absorbance at 450 nm, with a reference wavelength set between 540 and 590 nm
- Reagents reservoirs
- Distilled water

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- Calibrated pipettes (5-1000 µL)
- Calibrated multichannel pipettes (30-300 µL)
- Beakers, flasks, cylinders, tubes and liquid containers necessary for preparation of reagents
- Standard laboratory materials and equipment
- Software for regression analysis calibration curve and calculation of sample concentrations
- Optional: ELISA plate washer (compatible with 96-well plates)
- Optional: U bottom plate

Specimen Collection and Handling

Cell culture supernatant should be collected and handled according to standard cell culture procedures, see also IFU MAT Cell Set (M2016) and MAT Cell Set HS (M2017).

Test Principle

The PeliKine Human IL-6 ELISA MoAb is a "sandwich-type" of enzyme immunoassay. Samples, containing IL-6 (i.e. cell culture supernatant), together with a monoclonal 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 bound by the capturing and 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 colored 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.

Calibrators and Controls

The IL-6 standard (M191603) is calibrated to the 2nd International Standard for Interleukin-6 21/308 from the National Institute for Biological Standards and Control (NIBSC).

Storage and Shelf-Life

PeliKine Human IL-6 ELISA MoAb Set A (M2021) should be stored directly upon arrival, and after use, at -32 to -18°C.

PeliKine Human IL-6 ELISA MoAb Set B (M2022) should be stored directly upon arrival, and after use, at 2-8°C.

Products should be stored upright in the original packaging.

When stored under the recommended conditions, the shelf-life, mentioned on the label, is guaranteed.

IL-6 standard (M191603), biotinylated monoclonal antibody (MoAb) (M202104) and HPE-dilution buffer (M1940) are resistant up to three freeze-thaw cycles.

Precautions and Warnings

- Research use only, not for use in diagnostic procedures. The PeliKine Human IL-6 ELISA MoAb 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 MoAb Set A (M2021) should be stored at -32 to -18°C.
- The PeliKine Human IL-6 ELISA MoAb Set B (M2022) 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.
- IL-6 standard, biotinylated MoAb contains Thiomersal (0.001% w/v).
- HPE dilution buffer contains Thiomersal (0.005% w/v).
- Wash buffer contains Thiomersal (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.

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- Prior to the assay, frozen supernatant/samples should be thawed as quickly as possible, e.g. in a 37°C incubator for approximately 20 minutes.
- 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.
- Salt crystals may appear in the 5x concentrated HPE buffer and 20x wash buffer. Before preparing the working-strength buffers, first warm the concentrated buffer BRIEFLY to 37°C in a water bath to dissolve the precipitate.
- HPE buffer contains EDTA, refer to the MSDS of M2021 for details
- Stop solution contains sulphuric acid, refer to the MSDS of M2022 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 laboratory regulations.
- For optimal performance of ELISA be sure that all pipets and systems are checked and under full maintenance service according to described procedures of the manufacturers.

Assay Procedure

Components marked with “^(a)” are part of the PeliKine Human IL-6 ELISA MoAb Set A (Ref M2021), components marked with “^(b)” are part of the PeliKine Human IL-6 ELISA MoAb Set B (Ref M2022).

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 must 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.
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.

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:
 - Label 7 tubes, one tube for each dilution: 2250, 750, 250, 83.3, 27.8, 9.3, 3.1 pg/mL (final concentrations in the ELISA plate are 450, 150, 50, 16.7, 5.6, 1.9, 0.6 pg/mL).
 - Prepare the highest dilution by pipetting IL-6 and working-strength dilution buffer into the tube labelled 2250 pg/mL, minimum 100 µL total volume, mix well.
(Example: If stock concentration is 4000 pg/mL, then 63 µL IL-6 standard is mixed with 49 µL HPE buffer)
 - Add 80 µL of working-strength dilution buffer to the other tubes.
 - Transfer 40 µL of the highest dilution into the second tube labelled 750 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.

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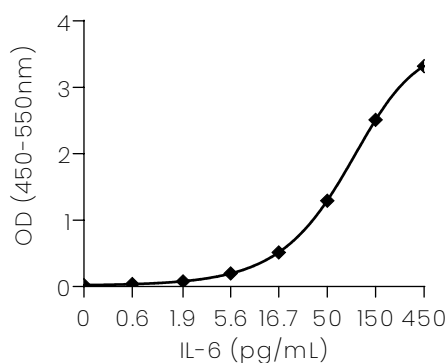
- Add 20 µL of the prepared IL-6 standard to columns 11 and 12 of the pre-coated plate and 20 µL working-strength HPE-dilution buffer to the blank wells (see also Figure 1).
 - Immediately add 80 µL working-strength biotinylated antibody to the wells, containing supernatant and standard curve, with a multichannel pipette and mix 3x gently by pipetting up and down; change the pipetting tips each row.
 - Cover the pre-coated plate with an adhesive 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 3 µL 10,000-fold concentrated streptavidin-poly-HRP conjugate^(a) to 30 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 an 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 8 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 8 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).

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

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

Results

A typical IL-6 standard curve is shown in Figure 2. This standard curve was generated for demonstration purposes only.



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Figure 2: Example of an IL-6 standard curve, fitted with five-parameter logistic non-linear regression.

Interpretation of Results

The IL-6 standard curve is valid when the parameters mentioned in the table below are met.

Parameter	Specification
Blank	< 0.04 Determined on the mean OD of blanks
Limit of Detection	< 1 pg/mL Determined by interpolation of mean blank OD + 3x st. dev.
Regression coefficient R²	> 0.98

Limitations

This ELISA kit has been designed for professional and research use only and for analysis of IL-6 in cell culture supernatant. This kit was validated with cell culture supernatant obtained from the MAT Cell Set (M2016) and MAT Cell Set HS (M2017). Supernatant from other cell or supplement sources or other matrices must be validated by the user.

The user must be trained and familiar with ELISA test procedures.

Only the washing steps were validated by Essange on an automated microplate washer (Biotek 405 TS). The other steps, as described in this IFU, were validated by Sanquin with a manual testing procedure. All claims in this IFU were validated with the manual testing procedure (except for the washing steps). When using the kit on an automated ELISA system, the test must be validated by the user before use. The claims in this IFU are not guaranteed for the use of this kit on an ELISA machine.

Rheumatoid Factor in the product may interfere with the ELISA read-out.

Analytical Performance

Performance Characteristic	Specification
Limit of Detection	< 1 pg/mL

Disclaimer

FOR RESEARCH USE ONLY,

Essange 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.

Changes to Previous Version

This is the first version, using template v1.