Sanquin

PeliKine™
human IL-6 ELISA kit

96 tests

An enzyme immunoassay
for the quantitative determination of human IL-6

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I. INTRODUCTION

Interleukin 6 (IL-6) is a mediator of the inflammatory response and is involved in the induction of acute phase proteins [1,2,3,4] and the development of fever [5]. A marked correlation between IL-6 levels and inflammatory processes has been demonstrated in synovial fluid and serum of rheumatoid arthritis patients [6,7,8] and in serum of patients with burns [9,10]. It was demonstrated that in recipients of kidney transplants the IL-6 levels in serum and urine hallmark the onset of rejection episodes [11,12]. Elevated IL-6 levels were also observed in sera of patients with septic shock, multiple myeloma and alcoholic hepatitis, and a significant difference between IL-6 levels of survivors and non-survivors was found [13,14,15].
Bioassays for the quantification of IL-6, based on the proliferation of B-cell hybridomas have been used for several years [16,17,18]. These assays, although sensitive, are time consuming and susceptible to interference by other substances.
This PeliKine™ huIL-6 ELISA kit [19] has been developed for faster, more reproducible and specific quantification of human IL-6 (huIL-6) in plasma and other body fluids, as well as in cell-culture supernatant.

II. PRINCIPLE OF THE TEST

The PeliKine™ huIL-6 ELISA kit is a "sandwich-type" of enzyme immunoassay in which a monoclonal anti-huIL-6 antibody has been precoated to polystyrene microtiter wells. HuIL-6, present in a measured volume of sample or standard, is bound by the antibody on the microtiter plate. Non-bound material is then removed by washing. Subsequently, a biotinylated sheep antibody to huIL-6 is added. This antibody binds to the huIL-6-antibody complex present in the microtiter well. Excess biotinylated antibody is removed by washing, followed by addition of horseradish peroxidase (HRP) conjugated streptavidine, which binds onto the biotinylated side of the huIL-6 sandwich. 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 huIL-6 present in the sample or standard. After the reaction has been terminated by the addition of a stop solution, absorbance is measured in a microtiter plate reader. From the absorbance of samples and those of a standard curve, the concentration of huIL-6 can be determined by interpolation with the standard curve. A schematic representation of this protocol and a proposed plate plan are given on the last pages of this leaflet.
The PeliKine™ huIL-6 ELISA kit is intended for research purposes only.

III. STORAGE AND STABILITY

At arrival the Pelikine™ human IL-6 ELISA kit will be stable for 3 months when kept at 2-8°C. Stability until the expiration date shown on the box label can be achieved by storing the anti-IL-6 biotin conjugate, and the streptavidin-poly-HRP conjugate separately at -18 to -32°C.

IV. CONTENTS OF THE KIT

The PeliKine™ human IL-6 ELISA kit contains material sufficient for 96 tests, including standard curve samples. The reagents provided are:

Quantity	Kit component		Volume	Cap colour
1 pieces	Precoated microplate	12 x 8 strips + plate-frame	-	
2 vial	Reombinant IL-6 standard (lyophilized)*	450 pg/ml	1000 µl**	-
1 vial	biotinylated antibody*	100-fold concentrated	150 µl	yellow
1 vial	streptavidin-poly-HRP conjugate*	10,000-fold concentrated	20 µl	brown
1 bottle	Wash buffer*	20-fold concentrated	50 ml	
1 bottle	HPE dilution buffer*	5- fold concentrated	55 ml	
1 bottle	TMB substrate solution	Ready-for-use	12.5 ml	brown
1 botle	Stop solution (0.18 M H_2SO_4	Ready-for-use	13.5 ml	white
5 pcs	plate seals	-	-	

* These reagents contain Merthiolate (0.001%) as a preservative.
** Volume after reconstitution of lyophilised material.

V. PRECAUTIONS FOR USE

1) The PeliKine™ human IL-6 ELISA kit is intended *for research purposes only.*
2) Only use the reagents and microtiter plates supplied with the kit, do not mix reagents from different kit lots.
3) Handle all plasma and serum samples with care to prevent transmission of blood-borne infections.
4) Sodium azide inactivates HRP, do not use sodium azide-containing solutions, nor add sodium azide to the supplied materials.
5) Centrifuge all vials before use (1 minute 3000 x g).
6) With the exception of the substrate blank wells, do not allow wells to stand uncovered or dry for extended periods between incubation steps.

VI. ADDITIONAL INFORMATION

Additional materials required

– Pipetting devices for accurate delivery of 1-10 µl, 50 µl, 100 µl and 1 ml volumes.
– Beakers, flasks, cylinders necessary for preparation of reagents.
– Device for delivery of washing buffer (wash bottle / automated plate washer).
– Microtiter plate reader.

Sensitivity

MEAN calculated zero signal + 3 SD : 0.3 pg/ml
2x (MEAN calculated zero signal) : 0.5 pg/ml

Expected values

IL-6 values in fresh serum and plasma of healthy individuals are below 10 pg/ml.

Specificity

No crossreactivity was observed with the following recombinant human proteins: IL-1α, IL-1ß, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, il-10, IL-11, IL-13, Macrophage Colony Stimulating Factor (M-CSF), Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF), Leukemia Inhibitory Factor (LIF), RANTES, Stem Cell Factor/ Mast Cell Factor (SCF/MCF), Transforming Growth Factor ß-1 (TGFß-1), Tumour Necrosis Factor α (TNFα), Tumour Necrosis Factor ß (TNFß/Lymphotoxin), and Interferon γ (IFNγ).

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VII. ASSAY PROCEDURE

- *BRING ALL REAGENT TO ROOM REMPERATURE (18-25°C),* with the exception of the streptavidin-HRP conjugate which has to be kept at -18°C to -32°C to ensure stability. Centrifugate all vials before use (1 minute 3000 x g).
- It is advised to test all samples and standard dilutions in duplicate.
- Mix all reagents thoroughly before use (without foaming).
- **For your convenience an easy-reference manual with check list and plate plan are available on the last pages of this leaflet.**

1. **MICROTITER PLATE** **Precoated strips**
   The kit contains one plate-frame with 12 strips of 8 wells, vacuum-sealed and packed in a resealable pouch. The PeliKine™ human IL-6 ELISA kit provides the flexibility to run two partial plates on separate occasions.

Before opening the plastic pouch, determine the number of strips required to test the desired number of samples plus 18 wells needed for running standards and blanks in duplicate. Remove non-used strips from the plate-frame and restore them in the plastic pouch containing the desiccant for up to 1 month at 2-8°C

2. **BUFFER PREPARATIONS**
   Wash buffer
   The kit contains one bottle with 20 fold concentrated wash buffer.

Prepare the wash buffer by adding 50 ml of the washbuffer concentrate (total content of the bottle) to 950 ml distilled water. The diluted washbuffer can be stored for up to 2 months at 2-8°C.

HPE-dilution buffer
The kit contains one bottle with 5-fold concentrated dilution buffer.
For optimal assay results, dilute samples and standard in working-strength dilution buffer.

Calculate the quantity of HPE-dilution buffer required (approximately 2 ml concentrated HPE buffer per microwell strip). Prepare a working-strength solution by diluting the opalescent concentrated buffer 5 fold in distilled water. The diluted buffer can be stored for up to one week at 2-8°C.

3. IL-6 standard **Standard curve preparation**

The kit contains two vials with 450 pg/ml of a lyophilized recombinant human IL-6 standard, calibrated against the WHO First International Standard (IL-6 89/548; National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, U.K. (1 WHO unit = 10 pg IL6, see ref [20])).

Reconstitute one vial by adding 1 ml of distilled water. Allow 10 minutes at room temperature to dissolve and mix gently. The reconstituted standard can be stored for two hours at 2-8°C and for 1 week between -18 to -32°C.
The second vial of lyophilized standard can be used in later assays.

Label 8 tubes, one tube for each dilution: 80, 32, 12.8, 5.1, 2.0, 0.8, 0.3 and 0 pg/ml.
Pipette 555 µl of working-strength dilution buffer into the tube labeled 80 pg/ml and 300 µl of working-strength dilution buffer into the other tubes.
Transfer 120 µl of the IL-6 standard (450 pg/ml) into the first tube labeled 80 pg/ml, mix well and transfer 200 µl of this dilution into the second tube labeled 32 pg/ml.
Repeat the serial dilution's six more times by adding 200 µl of the previous tube of diluted standard to the 300 µl of dilution buffer. The last tube contains only dilution buffer.

The standard curve will contain 80, 32, 12.8, 5.1, 2.0, 0.8, 0.3 and 0 pg/ml (dilution buffer).

It is recommended to prepare two separate series for each assay.

Avoid repeated freeze-thawing of the standard, although experimental data have shown that up to 3 freeze-thaw cycles have no effect on the IL-6 levels of the standard. Thaw the reconstituted standard in tap water (18-25°C), do not use 37°C or 56°C water baths for this purpose.

4. SAMPLES

Serum, EDTA-anti-coagulated plasmas, and culture fluids are suitable for use in the assay (caution: separate plasma/serum and blood cells within 4 hours after collection, non-separated samples must be kept on temperatures from 2 to 8°C). Do not use grossly haemolyzed or lipemic specimens. If samples are to be run within 24 hours, they may be stored at 2-8°C; otherwise samples should be stored frozen (between -18 to -32°C, preferably < -70°C).
Up to 3 freeze-thaw cycles have no effect on the IL-6 levels of serum or plasma samples. Nonetheless, excessive freeze-thaw cycles should be avoided. Prior to the assay, frozen samples should be thawed as quickly as possible in tap water (18-25°C), do not use a 37°C or 56°C water bath for this purpose.

It is recommended to dilute the test samples at least 1:2 in working-strength dilution buffer. If high levels of IL-6 (> 75 pg/ml) are expected in the test samples, additional dilutions of sample i.e. 1:10 and 1:50 should also be prepared.

5. **FIRST WASH STEP**

Prepare washing buffer as described on page 4 of this leaflet.

Wash the required microtiter plates five times with washing buffer in a plate washer.
In case of manual washing, completely fill the wells (> 300 µl) with washing buffer and aspirate, repeat this four times. After the final aspiration the wells should be dry.

6. **FIRST INCUBATION STEP** **Standards and samples**

Leaving the substrate blank wells empty, transfer 100 µl of the prepared standards and samples in duplicate into the appropriate wells (see recommended plate plan).
Cover plate(s) with adhesive seal, gently agitate the microtiter plate by tapping the edge of the plate for a few seconds to mix contents of each well and incubate for 1 hour at room temperature (18-25°C).

Just before washing, prepare next incubation reagent as described in point 8

7. **SECOND WASH STEP**

Aspirate supernatant from wells and wash the microtiter plate as described in point 5 above.

8. **SECOND INCUBATION STEP** **biotinylated antibody**

The kit contains one yellow-capped vial with biotinylated antibody.

Calculate the required amount of conjugate (10 µl per strip) and dilute 1:100 in HPE-dilution buffer.

Leaving the substrate blank wells empty, add 100 µl of diluted biotinylated antibody to all wells.

Cover plate(s) with adhesive seal, gently agitate the microtiter plate by tapping the edge of the plate for a few seconds to mix contents of each well and incubate for 1 hour at room temperature (18-25°C).

Just before washing prepare next incubation reagents as described in point 10.

9. **THIRD WASH STEP**

Aspirate supernatant from wells and wash the microtiter plate as described in point 5 above.

10. **THIRD INCUBATION STEP** **Streptavidin-HRP conjugate**

The kit contains one brown vial of concentrated streptavidin-HRP conjugate, which must be stored at –18°C to –32°C to maintain maximal stability. The contents of the vial will not be frozen at this temperature.

Per microtiter plate, add 3 µl streptavidin-HRP conjugate to 30 ml of working-strength dilution buffer just before use. Do not prepare in advance of assay.

Leaving the substrate blank wells empty, add 100 µl of streptavidin-HRP conjugate to all wells.

Cover the microtiter plate(s) with adhesive seal, agitate the microtiter plate by tapping the edge of the frame for a few seconds to mix contents of each well and incubate for 30 minutes at room temperature (18-25°C).

11. **FOURTH WASH STEP**

Aspirate supernatant from wells and wash the microtiter plate as described in point 5 above.

12. **FOURTH INCUBATION STEP** **Enzymatic colour development**

The kit contains one brown-capped bottle with a ready-for-use TMB substrate solution containing a mixture of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide.
Protect from prolonged exposure to light.
This solution must be on room temperature before use!

Add 100 µl of substrate solution to all wells, **including the substrate blank wells**.

Cover microtiter plate(s) with lid, gently agitate the microtiter plate by tapping the edge of the frame for a few seconds to mix contents of each well and **incubate for 30 minutes at room temperature (18-25°C) in the dark.**
Do not cover the plate with aluminum foil.

13. **STOP ENZYMATIC REACTION**

The kit contains one white-capped bottle with a ready-for-use stop solution of 0.18 M H_2SO_4.

Add 100 µl of stop solution to all wells.

After stopping the colour is stable for maximally 30 minutes.

14. **PLATE READ-OUT**

Read at 450 nm in an ELISA reader.

VIII. RESULTS

Substrate blank

- Record the absorbance at 450 nm for the substrate blank wells and average the duplicate values.

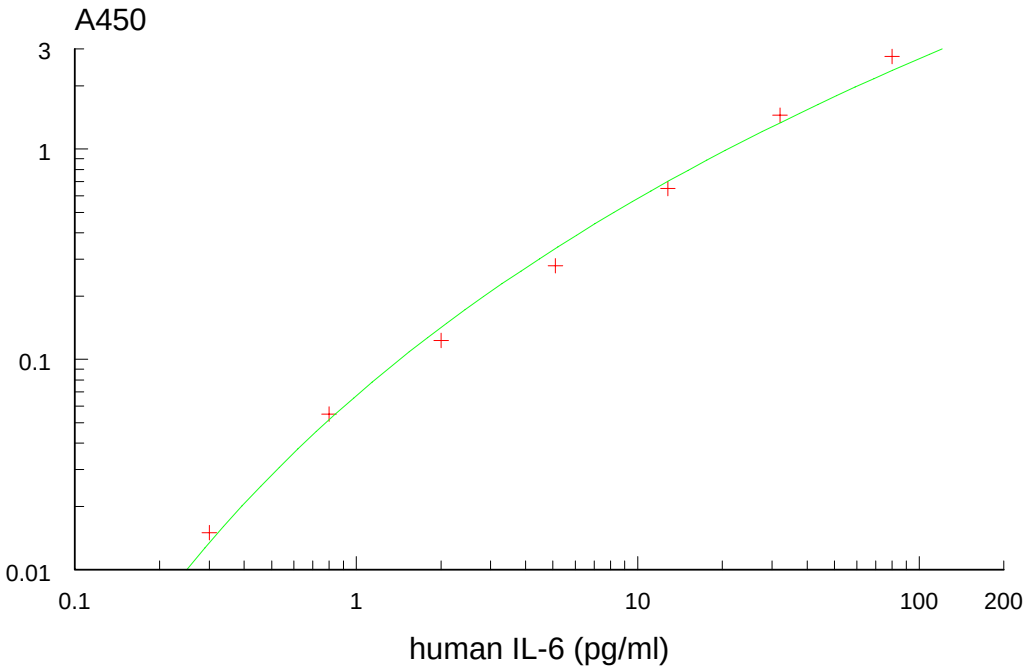
Standard curve

- Record the absorbance at 450 nm for each well containing standard and average the duplicate values.
- Calculate the net average absorbances by subtracting the average of the substrate blank wells.
- Plot the net average absorbances (ordinate) versus the IL-6 concentration in pg/ml (abscissa) on log-linear paper and draw the best fitting curve. An example of a standard curve is given on the next page.

Samples

- Record the absorbance at 450 nm for each standard well, and average the duplicate values.
- Calculate the net average absorbances by subtracting the average of the substrate blank wells.
- Locate the net average absorbance value found for each sample on the vertical axis and follow a horizontal line intersecting the standard curve. At the point of intersection, read the IL-6 concentration (pg/ml) from the horizontal axis. Multiply the obtained IL-6 concentration with the dilution factor of the sample and record this figure.

A computer program to calculate ELISA results (developed by Mr E.J. Nieuwenhuys, CLB Amsterdam) is available free of charge to our kit users.
This program can be downloaded from internet:
http://www.xs4all.nl/~ednieuw/Calibration/Logit/logit.htm



Typical standard curve for the PeliKine™ human IL-6 ELISA kit

concentration huIL-6 (pg/ml)	Calculated mean absorbance at 450 nm
substrate blank	0
0 pg/ml	0.021
0.3 pg/ml	0.015
0.8 pg/ml	0.055
2.0 pg/ml	0.123
5.1 pg/ml	0.279
12.8 pg/ml	0.650
32 pg/ml	1.450
80 pg/ml	2.754

DO NOT USE THESE DATA TO CONSTRUCT A STANDARD CURVE FOR SAMPLE VALUE CALCULATIONS

Plate plan proposed for the PeliKine™ human IL-6 ELISA kit:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1										
B	S2	S2										
C	S3	S3										
D	S4	S4										
E	S5	S5										
F	S6	S6										
G	S7	S7										
H	S8	S8									B	B

Key: B: substrate blank S1-S8: IL-6 standards 0-80 pg/ml Empty: samples

Protocol summery and checklist PeliKine™ human IL-6 ELISA kit

- O Bring all reagents, with the exception of streptavidin-HRP, to room temperature.
- O Prepare dilution buffer.
- O Prepare standard and sample dilutions.
- O Prepare washing buffer.
- O Wash the plate five times with washing buffer.
- O Leaving the substrate blank wells empty, add 100 μl of standard and sample dilutions to the appropriate wells, cover the plate and incubate for one hour at room temperature.
- O Dilute biotinylated antibody 1:100 in dilution buffer.
- O Leaving the substrate blank wells empty, add 100 μl of the diluted biotinylated antibody to all wells, cover the plate and incubate for one hour at room temperature.
- O Dilute the streptavidin-HRP conjugate 1:10,000 in dilution buffer.
- O Wash the plate five times with washing buffer.
- O Leaving the substrate blank wells empty, add 100 μl of the streptavidin-HRP conjugate to all wells, cover the plate and incubate for 30 minutes at room temperature.
- O Wash the plate five times with washing buffer.
- O Add 100 μl substrate solution to all wells, including the substrate blank wells, incubate for 30 minutes at room temperature in the dark.
- O Add 100 μl stop solution to all wells and read the plate at 450 nm.
- O Calculate the amount of IL-6 in the samples.