PeliKine™
human IL-4 ELISA kit

96 tests

An enzyme immunoassay
for the quantitative determination of human IL-4

PRODUCT INFORMATION

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I. INTRODUCTION

Interleukin 4 (IL-4, B-cell growth factor-1, BSF-1) is a T-cell-derived cytokine with a molecular weight of approximately 15 to 19 kD. It plays an important role in the activation of resting B-cells and the subsequent proliferation and differentiation of B-cells [1-4].
IL-4 is essential for IgE synthesis *in vitro* [5] and the involvement with allergic diseases has been investigated [6,7]. Furthermore, IL-4 was found to inhibit the secretion of IL-1β, TNF-α and IL-6 of human monocytes [8], to down-regulate the surface expression of CD5 on B-cells [9] and to promote the growth of human T-cells [10]. Also anti-inflammatory effects of IL-4 have been reported [11]. Several studies have been conducted to assess the role of IL-4 in several autoimmune diseases [12-15].
IL-4 is produced by $CD4^+$ TH0 and TH2 cells [16-17], $CD8^+$ T-cells [18], fetal thymocytes [19], basophils [20] and mast cells [21, and reviews 22-25].
Bioassays for the quantification of IL-4, based on T-cell proliferation have been used for several years [26]. These assays, although sensitive, are time consuming and susceptible to interference by other substances.
This IL-4 ELISA [27] has been developed for fast, reproducible and specific quantification of human IL-4 (huIL-4) in plasma and serum as well as in cell-culture supernatant.

II. PRINCIPLE OF THE TEST

The PeliKine™ human IL-4 ELISA kit is based on a "sandwich-type" of enzyme immunoassay in which polystyrene microwell strips are coated with a monoclonal antibody to human IL-4. Human IL-4, present in the sample or standard, is captured by the antibody on the microtiter well, and non-bound material is removed by washing. Subsequently, a biotin-conjugated antibody to IL-4 is added, which binds to the IL-4-antibody complex present in the well. Excess biotinylated antibody is removed by washing, followed by the addition of polymerized horseradish peroxidase (poly-HRP), which is conjugated to streptavidin. After washing, a TMB-substrate solution is added to the wells and a coloured product is formed in proportion to the amount of IL-4 present in the sample or standard. The reaction is terminated by the addition of a stop solution; subsequently absorbance is measured in a microplate reader. By comparison of the absorbance of the samples to those of the standard curve, the concentration of IL-4 can be determined.
As a special feature, all dilutions are prepared in High Performance ELISA (HPE) dilution buffer, which allows the assay to be performed in different matrices.

III. STORAGE AND STABILITY

At arrival the Pelikine™ human IL-4 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-4 biotin conjugate, and the streptavidin-poly-HRP conjugate separately at -18 to -32°C°C.

IV. CONTENTS OF THE KIT

The PeliKine™ human IL-4 ELISA kit contains sufficient reagents for 96 tests, including standard curve samples. The reagents provided are:

Quantity	Kit component		Volume	Cap colour
1 pc	precoated microplate	12 x 8 strips + plate-frame	-	
2 vials	recombinant IL-4 standard*	5850 pg/ml	200 µl	-
1 vial	anti-IL-4 biotin conjugate*	100-fold concentrated	150 µl	yellow
1 vial	streptavidin-poly-HRP*	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 bottle	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% w/v) as a preservative.
** Volume after reconstitution of lyophilised material.

VI. PRECAUTIONS FOR USE

1) The PeliKine™ human IL-4 ELISA kit is intended *for research purposes only*.
2) Only use the reagents and microtiter plate supplied with the kit, do not mix reagents from different production 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.

V. ADDITIONAL INFORMATION

Additional materials required

- Pipetting devices for accurate delivery of 1-10 µl, 50 µl, 100 µl and 1 ml volumes.
- Beakers, flasks and cylinders 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 : < 0.7 pg/ml
2 x (MEAN calculated zero signal) : < 1.0 pg/ml

Expected values

IL-4 values in fresh serum and plasma samples of healthy individuals are below 0.4 pg/ml.

Specificity

No crossreactivity was observed with the following recombinant human proteins: IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, Macrophage Colony Stimulating Factor (M-CSF), Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF), Leukaemia Inhibitory Factor (LIF), RANTES, Stem Cell Factor/ Mast Cell Factor (SCF/MCF), Transforming Growth Factor β-1 (TGFβ-1), Tumour Necrosis Factor α (TNFα), Tumour Necrosis Factor β (TNFβ/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**
   The kit contains one plate-frame with 12 strips of 8 wells, vacuum-sealed and packed in a resealable pouch. The PeliKine human IL-4 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 wash buffer concentrate (total content of the bottle) to 950 ml distilled water. The diluted wash buffer 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-4 standard**
The kit contains two vials with 5850 pg/ml of a recombinant human IL-4 standard, calibrated against the WHO First International Standard (IL-4 88/656; National Intitute for Biological Standards and Control, Potters Bar, Hertfordshire, U.K. (1 WHO Unit = 100 pg IL4.))

Label 8 tubes, one tube for each dilution: 180, 72, 28.8, 11.5, 4.6, 1.8, 0.7 and 0 pg/ml.
Pipette 630 µl of working-strength dilution buffer into the tube labeled 180 pg/ml) and 150 µl of working-strength dilution buffer into the other tubes.
Transfer 20 µl of the IL-4 standard (5850 pg/ml) into the first tube labeled 180 pg/ml, mix well and transfer 100 µl of this dilution into the second tube labeled 72 pg/ml.
Repeat the serial dilution's six more times by adding 100 ul of the previous tube of diluted standard to the 150 ul of dilution buffer. The last tube contains only dilution buffer.

The standard curve will contain 180, 72, 28.8, 11.5, 4.6, 1.8, 0.7 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 op to 3 freeze-thaw cycles have no effect on the IL-4 levels of the standard. Thaw the 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 (<-18°C, preferably <-70°C). Avoid freezing and thawing samples more than once. 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 samples at least 1:2 in working-strength dilution buffer.
If high levels of IL-4 (>180 pg/ml) are expected in the test samples, additional dilutions of the 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 microwells 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 ul 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 the wells and wash the plate as described in point 5.

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 diluted biotinylated conjugate to all wells.

Cover plate(s) with adhesive seal, gently agitate the 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 microtiterplate as described in point 5.

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 ul streptavidin-HRP conjugate to 30 ml of working-strength dilution buffer just before use. Do not prepare in advance of assay.

Leaving the substrate wells empty, add 100 µl of diluted 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 WASHSTEP**

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

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 the 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 or adhesive 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.

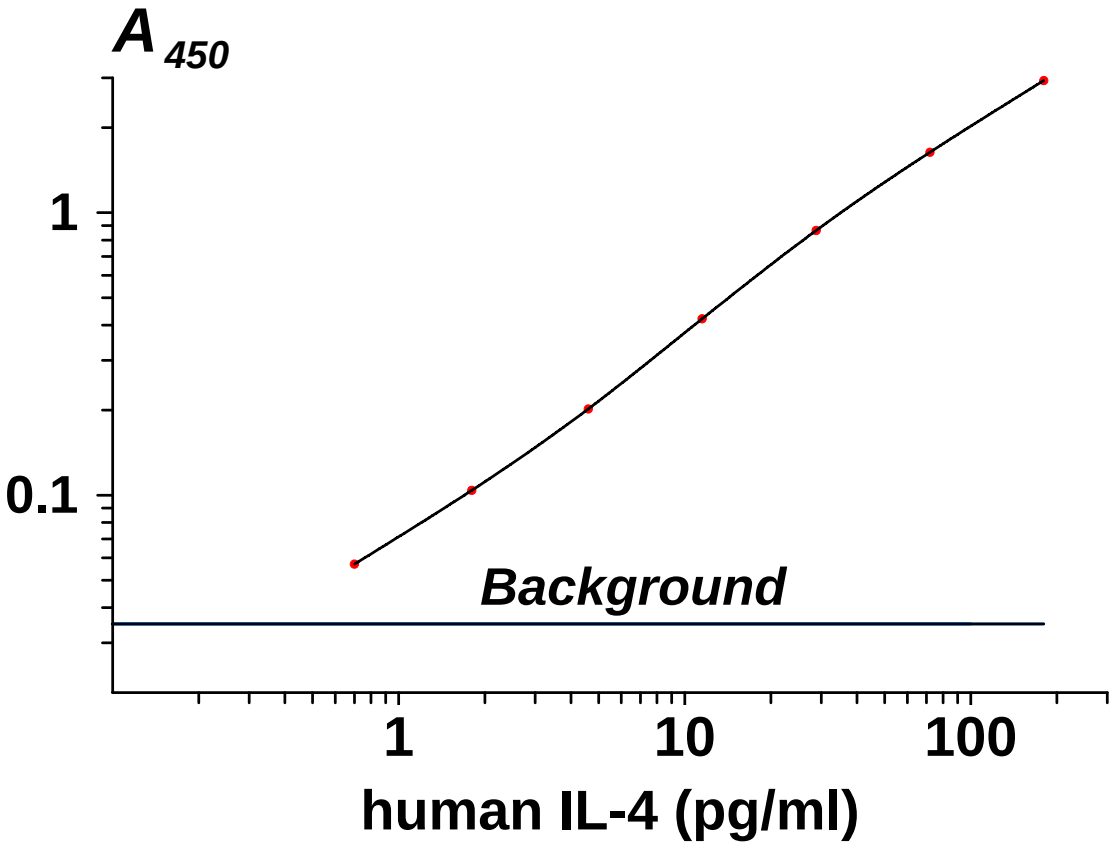
Standard curve

- 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-4 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 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 ordinate and follow a horizontal line intersecting the standard curve. At the point of intersection, read the IL-4 concentration (pg/ml) on the abscissa. Multiply the obtained IL-4 concentration by the dilution factor of the sample and record this figure.

A computer program to calculate ELISA results (developed by Mr E.J. Nieuwenhuys, Sanquin 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-4 ELISA kit

concentration hulL-4 (pg/ml)	Calculated mean absorbance at 450 nm
substrate blank	0
0	0.035
0.7	0.057
1.8	0.104
4.6	0.202
11.5	0.421
28.8	0.865
72	1.637
180	2.937

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

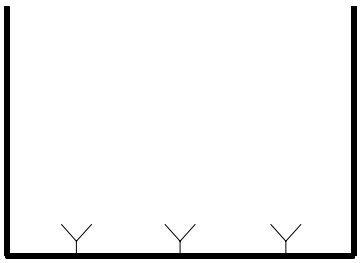
Protocol summary and checklist

O Bring all reagents to room temperature.

O Prepare HPE-dilution buffer.

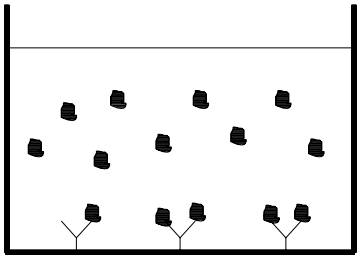
O Prepare standard and sample dilutions.

O Prepare washing buffer.



O Wash the plate five times with washing buffer.

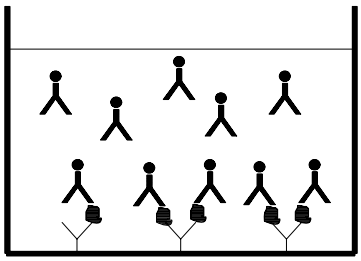
O Add 100 µl of standard and sample dilutions to the appropriate wells, leaving the substrate blank wells empty, cover the plate and incubate for one hour at room temperature.



O Dilute biotin-conjugated anti-IL-4 antibody 1:100 in HPE-dilution buffer.

O Wash the plate five times with washing buffer.

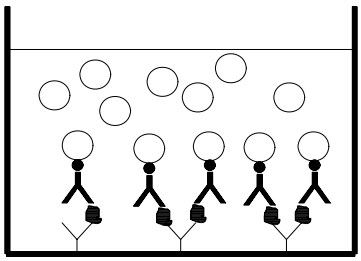
O Add 100 µl of the diluted biotin-conjugated anti-IL-4 antibody to all wells, leaving the substrate blank wells empty, cover the plate and incubate for one hour at room temperature.



O Dilute the streptavidin-poly-HRP conjugate 1:10,000 in HPE-dilution buffer.

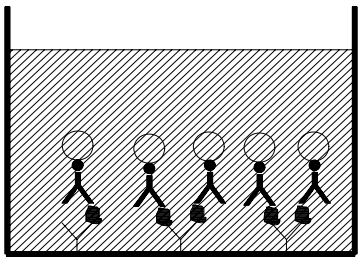
O Wash the plate five times with washing buffer.

O Add 100 µl of the diluted streptavidin-poly-HRP conjugate to all wells, leaving the substrate blank wells empty, cover 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, and 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-4 in the samples.

Key to the figures

Precoated monoclonal antibody to IL-4

IL-4 present in testsample

biotin conjugated antibody to IL-4

Streptavidine-Poly-HRP

Plate plan proposed for the Pelikine™ human IL-4 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-4 standards 0 - 180 pg/ml Empty: samples