



# **PeliKineä**

## **human IL-4 ELISA kit**

96 tests

An enzyme immunoassay for the quantitative determination  
of human Interleukin 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-1 $\beta$ , TNF- $\alpha$  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<sup>+</sup> TH0 and TH2 cells [16-17], CD8<sup>+</sup> 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 (hull-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

When kept at 2-8°C the Pelikine™ human IL-4 ELISA kit will be stable for 6 months. Stability until the expiration date shown on the box label can be achieved by storing the biotin-conjugated antibody to IL-4, and the streptavidin-poly-HRP conjugate separately below -18°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
1 pc	precoated microplate 12 x 8 strips + plate-	-
1 vials	recombinant IL-4 standard*	5850 pg/ml 200 $\mu$ l
1 vial	anti-IL-4 biotin conjugate*	100-fold concentrated 150 $\mu$ l
1 vial	streptavidin-poly-HRP*	10,000-fold concentrated 20 $\mu$ l
1 bottle	wash buffer*	20-fold concentrated 50 ml
1 bottle	HPE dilution buffer*	5-fold concentrated 60 ml
1 bottle	TMB substrate solution	ready-for-use 12.5 ml
1 bottle	stop solution (0.18 M H <sub>2</sub> SO <sub>4</sub> )	ready-for-use 13.5 ml
5 pcs	plate seals	-

\* These reagents contain Thiomersal<sup>R</sup> as a preservative (see VI,5).

\*\* Volume after reconstitution of lyophilised material.

#### V. ADDITIONAL MATERIALS REQUIRED

- Pipetting devices for accurate delivery of 1-10  $\mu$ l, 50  $\mu$ l, 100  $\mu$ l and 1 ml volumes.
- Distilled or de-ionised water.
- Glass tubes for making dilutions.
- Beakers, flasks and cylinders for preparation of reagents.
- Device for delivery of washing buffer (wash bottle / automated plate washer).
- Microplate reader, capable of measuring absorbance at 450 nm.

#### VI. PRECAUTIONS

- 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) Reagents containing Thiomersal<sup>R</sup> may be toxic upon ingestion, inhalation or skin contact. Avoid contact of skin, eyes or clothing with dilution, washing or substrate buffer. In case of contact, wash skin or eyes with water and consult a physician.
- 6) With the exception of the substrate blank wells, do not allow wells to stand uncovered or dry for extended periods between incubation steps.

## VII. ASSAY PROCEDURE

- Bring all reagents to room temperature (18-25°C)  
Please follow this handy step-by-step protocol in the indicated order. For your convenience, a checklist and plate plan are shown on pages 10 and 11.
- It is advised to test all samples and standard dilutions in duplicate.
- Mix all reagents thoroughly before use (without foaming).

### 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

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

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. PREPARATION OF STANDARD CURVE

A recombinant huIL-4 standard has been calibrated against the WHO First International Standard (IL-4 88/656; National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, U.K. (1 WHO Unit = 100 pg IL4.))

The kit contains one black-capped vial of 5850 pg/ml huIL-4.

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 HPE-dilution buffer into the first tube (180 pg/ml) and 150 µl to the other tubes.

Pipette 20 µl of the IL-4 standard (5850 pg/ml) into the first tube (180 pg/ml) and mix well. Transfer 100 µl of this dilution into the next tube (72 pg/ml) and continue to obtain serial dilutions.

The last tube (0 pg/ml) contains only HPE-dilution buffer.

### 4. PREPARATION OF 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 37°C or 56°C water baths for this purpose.

**Dilute the samples at least 1:2 in HPE-dilution buffer.**

If IL-4 levels of > 180 pg/ml are expected, the sample should be diluted such that the concentration will be within the assay range.

## 5. FIRST WASHING STEP

**Wash the required microwells in the plate-frame five times with washing buffer.**

For manual washing, completely fill the wells (> 300 µl) with washing buffer and discard, repeat this procedure four times. Finally the wells should be completely empty: Subsequent reagent should be added immediately, do not let the wells stand dry for extended period of time.

## 6. INCUBATION WITH STANDARDS AND SAMPLES

**Add 100 µl of the diluted standards and samples into the appropriate wells, leaving the substrate blank wells empty** (see recommended plate plan on page 11).

Cover plate with adhesive seal, gently agitate the microtiter plate by tapping the edge for a few seconds to mix contents of each well.

**Incubate for 1 hour at room temperature (18-25°C).**

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

## 7. SECOND WASHING STEP

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

## 8. INCUBATION WITH BIOTIN-CONJUGATED ANTIBODY TO IL-4

The kit contains one yellow-capped vial with concentrated biotin-conjugated antibody to IL-4.

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

**Add 100 µl diluted conjugate to the wells, leaving the substrate blank wells empty.**

Cover plate with adhesive seal, gently agitate the plate by tapping the edge for a few seconds to mix.

**Incubate for 1 hour at room temperature (18-25°C).**

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

## 9. THIRD WASHING STEP

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

## 10. INCUBATION WITH STREPTAVIDIN-POLY-HRP

The kit contains one brown-capped vial of concentrated streptavidin-poly-HRP, 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.

Dilute the concentrated streptavidin-poly-HRP 1:10,000 in HPE-dilution buffer. For this dilution we advise to pipette 3 µl of concentrated streptavidin-poly-HRP in 30 ml HPE-dilution buffer (regardless the amount of wells to be used).

**Add 100 µl of diluted streptavidin-poly-HRP conjugate to the wells, leaving the substrate blank wells empty.**

Gently agitate the plate by tapping the edge for a few seconds to mix contents of each well.

**Incubate for 30 minutes at room temperature (18-25°C).**

## 11. FOURTH WASHING STEP

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

## 12. INCUBATION WITH TMB SUBSTRATE

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 roomtemperature before use!**

**Add 100 µl of substrate solution to all wells, including the substrate blank wells.** Cover the microplate, gently agitate by tapping the edge for a few seconds to mix contents of each well.

**Incubate for 30 minutes at room temperature (18-25°C) in the dark.**

*Do not cover the plate with aluminium 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<sub>2</sub>SO<sub>4</sub>.

**Add 100 µl of stop solution to all wells.**

### 14. PLATE READ-OUT

Read **within 30 minutes** 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.

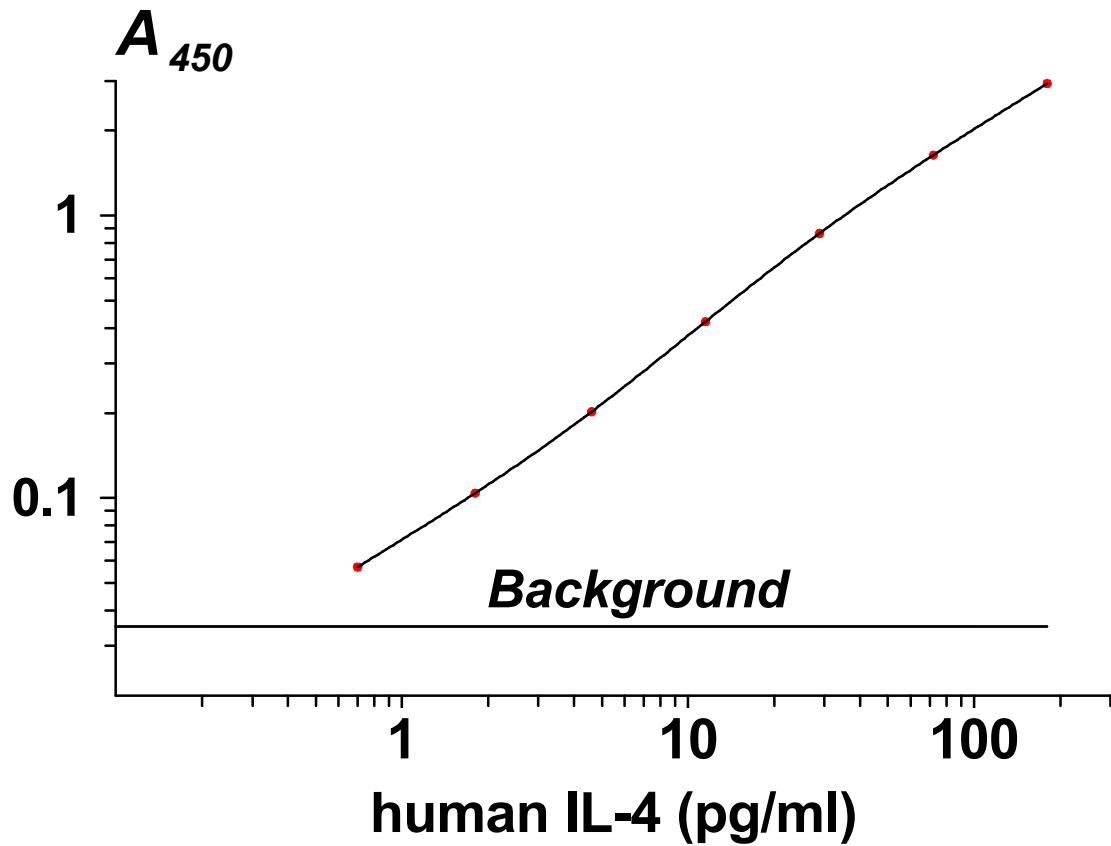
### Samples

- 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/LOGIT.HTM](http://www.xs4all.nl/~ednieuw/logit.htm) (download: regres.zip).



Typical standard curve for the PeliKine™ human IL-4 ELISA kit

concentration huIL-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

## IX. FEATURES

### 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

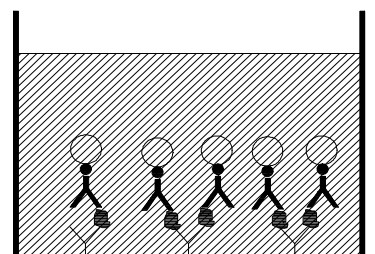
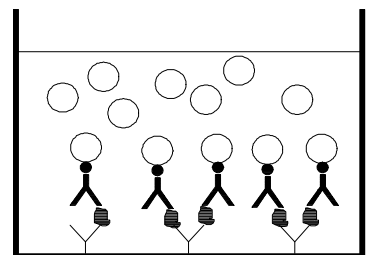
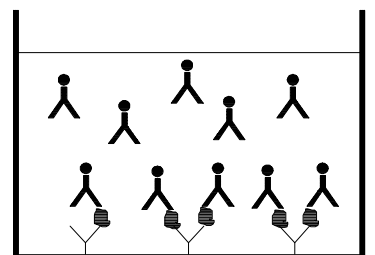
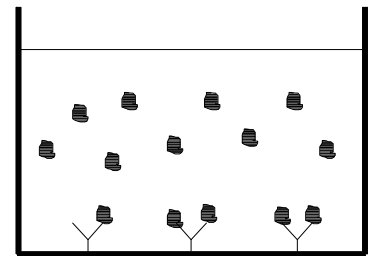
No crossreactivity was observed with the following recombinant human proteins: IL-1a, IL-1 $\beta$ , IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, Macrophage Colony Stimulating Factor (M-CSF), Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF), Leukaemia Inhibitory Factor (LIF), RANTES, Stem Cell Factor/ Mast Cell Factor (SCF/MCF), Transforming Growth Factor  $\beta$ -1 (TGF $\beta$ -1), Tumour Necrosis Factor  $\alpha$  (TNF $\alpha$ ), Tumour Necrosis Factor  $\beta$  (TNF $\beta$ /Lymphotoxin), and Interferon  $\gamma$  (IFN $\gamma$ ).

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**Protocol summary and checklist**

- Bring all reagents to room temperature.
- Prepare HPE-dilution buffer.
- Prepare standard and sample dilutions.
- Prepare washing buffer.
- Wash the plate five times with washing buffer.
- 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.
- Dilute biotin-conjugated anti-IL-4 antibody 1:100 in HPE-dilution buffer.
- Wash the plate five times with washing buffer.
- 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.
- Dilute the streptavidin-poly-HRP conjugate 1:10,000 in HPE-dilution buffer.
- Wash the plate five times with washing buffer.
- 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.
- Wash the plate five times with washing buffer.
- Add 100 µl substrate solution to all wells, including the substrate blank wells, and incubate for 30 minutes at room temperature in the dark.
- Add 100 µl stop solution to all wells and read the plate at 450 nm.
- Calculate the amount of IL-4 in the samples.

**Key to the figures**

- Y Precoated monoclonal antibody to IL-4
- IL-4 present in testsample
- 人 biotin conjugated antibody to IL-4
- Streptavidine-Poly-HRP

Plate plan proposed for the Pelikine™ human IL-4 ELISA kit:

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

Key: B: substrate blank S1-S8: IL-4 standards 0 - 180 pg/ml Empty: samples