

## IL-6 ELISA

Cat. No. 770021

IVD

The DiaMed EuroGen IL-6 ELISA is an Enzyme Immunoassay for the quantitative determination of IL-6.

### CLINICAL SIGNIFICANCE

Human IL-6 is a 26 kD polypeptid, secreted by a variety of cells including activated T-lymphocytes, monocytes, fibroblasts, endothelial cells and keratinocytes. The production of IL-6 is induced by various infections and inflammatory stimuli e.g. IL-1, bacterial LPS, virus infection, UV-light and others. IL-6 displays a very pleiotropic action on a broad range of target cells involved in the immune system, hematopoiesis, inflammation and reproduction. These multiple actions are integrated in a network of cytokine interactions where the final effect results from synergistic or antagonistic activities between IL-6 and other cytokines. Depending on the target cell population IL-6 may have growth stimulating properties as well as differentiating capacity.

The mechanism by which this cytokine can mediate multiple functions is complex and there is evidence that at least two membrane proteins are involved in this process, the IL-6 receptor and a 130 kD glycoprotein.

Among the biological effects in which IL-6 has been reported to be involved in are the induction of a final maturation of B-cells, induction, growth and cytotoxic differentiation of T-cells, stimulation of colony forming cells in hematopoiesis, maturation of megakaryocytes with increase of the number of platelets in vivo, induction of growth of plasmacytoma and myeloma cells, growth factor capacity for hybridoma cells and Epstein-Barr Virus immortalized B-cells, induction of neural differentiation, growth inhibition and induction of differentiation of myeloid leukemic cell lines into macrophages and finally the regulation of the acute response in hepatocytes. In summary, IL-6 may be considered to be an important mediator in host defense mechanisms by affecting antibody production, hematopoiesis and the acute-phase response.

### PRINCIPLE

Microtiterstrips coated with anti-IL-6 monoclonal antibodies are incubated with standard sera and patient samples. During this incubation, IL-6 is bound to the immobilized antibodies. After removal of the unbound material by a washing procedure, the IL-6 is bound in a second incubation step to a second IL-6 specific monoclonal antibody which is conjugated to biotin. After the removal of excess biotin-conjugate by a washing procedure, the amount of biotin-conjugated monoclonal antibody is detected in a third reaction step by the addition of streptavidin-HRP. After the removal of unbound HRP-conjugate, the microtiterstrips are incubated with a chromogen solution containing hydrogen peroxide and tetramethylbenzidine. A blue colour develops in proportion to the amount of IL-6 bound to the wells of the microtiterstrips. The enzymatic reaction is stopped by the addition of 2N H<sub>2</sub>SO<sub>4</sub> and the absorbance values at 450 nm are determined. A standard curve is obtained by plotting the absorbance value of the IL-6 standards versus the corresponding standard value (pg/ml). The concentration of IL-6 in patient samples is determined by interpolation from this standard curve.

### REAGENTS

**1a-f. Standard Sera:** 5 vials (a-e), containing 1.0 ml of a human serum based protein matrix with resp. 10-25-50-200 and 500 pg/ml IL-6. Sera contain 0.09 % NaN<sub>3</sub> as preservative. 1 vial (f), containing +/- 1 ng lyophilised IL-6, for the determination of IL-6 in cell culture supernatans or non-serum samples (e.g. spinal fluid, urine, synovial fluid, etc...). The exact concentration is indicated on the vial. The standards are calibrated against the "Unclassified Interleukin-6 (recDNA human type) 89/548".

**2. Zero Standard or Sample Diluent:** 1 vial, containing 5 ml of a human serum protein matrix. Serum contains 0.1 % NaN<sub>3</sub> as preservative.

**3. Coated Microtiterstrips:** 1 plate (12 x 8-well strips) coated with monoclonal antibodies to IL-6.

**4. Washing Buffer:** 1 vial, containing 100 ml 20 x concentrated phosphate-buffered washing solution. Preservatives: antimicrobial agents.

**5. Biotin-anti-IL-6 Conjugate:** 1 vial, containing 15 ml biotinylated monoclonal antibodies to IL-6. Contains antimicrobial agents and an inert red dye.

**6. Streptavidin-HRP Conjugate:** 1 vial, containing 15 ml streptavidin-HRP. Contains antimicrobial agents and an inert red dye.

**7. Chromogen Solution:** 1 vial, containing 25 ml of a solution containing H<sub>2</sub>O<sub>2</sub> and tetramethyl-benzidine.

**8. Stopping Solution:** 1 vial, containing 12 ml 2 N H<sub>2</sub>SO<sub>4</sub>.

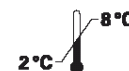
### MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision micropipettes with disposable tips.
2. Clean standard laboratory volumetric glassware.
3. Microtiterplate reader capable of measuring absorbances at 450 nm.

### WARNINGS AND PRECAUTIONS FOR USERS

1. For in vitro diagnostic use only.
2. Human blood components used in the preparation of the standard sera have been tested and found to be nonreactive for hepatitis B surface antigen and HIV. Since no known method can ever offer complete assurance that products derived from human blood will not transmit hepatitis or other viral infections, it is recommended to handle these control sera as potentially infectious material. Dispose of patient samples and all materials used to perform this test as if they contain infectious agents.
3. Do not exchange conjugates or coated microtiterstrips from kits with different lot numbers.
4. Some kit components contain sodium azide as a preservative. In order to prevent the formation of explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.

### STORAGE CONDITIONS



1. Do not expose chromogen solution to strong light or high temperature during storage. This solution should be colourless; if not, it should be replaced.
2. Store the microtiterstrips in their original bag with the desiccant until all strips have been used.
3. Never use any kit components beyond the expiration date.

### SPECIMEN COLLECTION AND PREPARATION

Human serum and plasma may be used in this assay. **It is very important to remove the serum from clot as soon as possible to avoid hemolysis.** Lipemic and/or hemolysed samples can cause erroneous results. Separation of the serum from the clot is also important to avoid false positive results due to possible release of IL-6 by the monocytes after the blood has been taken. Small amounts of LPS or other stimuli in the collection tube may interfere with the determination if the serum is not separated from the clot.

Transfer the serum to a clean storage tube. Specimens may be stored at 2-8°C for a few days, or they can be stored frozen for a longer period of time. Avoid repeated freezing and thawing.

### ASSAY PROCEDURE

#### General Remarks

1. Use a separate disposable tip for each transfer to avoid cross-contamination.
2. All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
3. Once the assay has been started, all steps should be completed without interruption.
4. Absorbance is a function of the incubation time and temperature. Therefore the size of the assay run should be limited. It is suggested to run no more than 20 patient samples with one set of Reference Standards in duplicate.

## Reconstitution of the Reagents

1. Washing Buffer: dilute 100 ml of concentrated Washing Buffer (4) to 2000 ml with distilled water. **Caution:** Crystals may form when concentrated washing solution is stored at 2-8°C. These crystals can easily be dissolved when bringing the vials to room temperature or by putting them in a water bath at 37°C.
2. Lyophilised IL-6: the lyophilised content of the vial f can be reconstituted in cell culture medium or alternative matrices.

## Assay Procedure

1. Place the desired number of strips (3) in the microtiterstrip holder.
2. Pipette 125 µl of the standard sera (2,1) and samples into each pair of adjacent wells.
3. Incubate the covered microtiterstrips in a moist atmosphere for  $20 \pm 2$  min at  $37 \pm 2^\circ\text{C}$ .
4. Invert the microtiterstrips over a suitable container and briskly shake out the contents. Immerse the strips immediately in the reconstituted Washing Solution. This washing step is performed 5 times. It is important to change the washing solution between the consecutive washing steps. During the first step, the washing solution is left in the strips for 2-3 min. Finally empty the microtiterstrips and remove excess fluid by blotting the inverted strips on absorbent paper. Alternatively an automatic device can be used.
5. Add 125 µl of biotin-anti-IL-6 Conjugate (5) and incubate the covered microtiterstrips in a moist atmosphere for  $20 \pm 2$  min at  $37 \pm 2^\circ\text{C}$ .
6. Repeat the washing cycle as described in 4.
7. Add 125 µl of streptavidin-HRP Conjugate (6) and incubate the covered microtiterstrips in a moist atmosphere for  $20 \pm 2$  min at  $37 \pm 2^\circ\text{C}$ .
8. Repeat the washing cycle as described in 4.
9. Add 100 µl of Chromogen Solution (7) to each well and incubate for  $20 \pm 2$  min at  $37 \pm 2^\circ\text{C}$ . Avoid light exposure during this incubation.
10. Add 50 µl of Stopping Solution (8) to each well.
11. Blank the microtiterplate reader and determine the absorbance of each well at 450 nm within 30 min following the addition of acid.

## RESULTS

### Calculation of the Results

Plot the average absorbance values obtained for each standard serum against the corresponding IL-6 concentration (pg/ml) and construct a calibration curve with an optimised curve fit (e.g. log/linear).

Use the average absorbance of each patient sample and determine the patient's IL-6 concentration by interpolation from this curve. Depending on the availability of computer capability, alternative methods of data reduction may be used.

### Expected values

The IL-6 concentrations in serum samples from 437 normally healthy Belgian blood donors were determined with the **DiaMed Eurogen IL-6 ELISA**. In this study 95 % of the subjects showed IL-6 values below 5 pg/ml.

## PERFORMANCE CHARACTERISTICS

### Precision

Intra-assay (n=10)	Pool A	Pool B
Mean (pg/ml)	71.2	382.1
S.D.(pg/ml)	3.9	35.9
C.V.(%)	5.5	9.4

### Inter-assay (n=7)

	Pool A	Pool B	Pool C
Mean (pg/ml)	35.9	54.8	230.4
S.D.(pg/ml)	2.4	4.8	8.8
C.V.(%)	6.8	8.7	3.8

### Sensitivity

The minimum detectable concentration (mean of 6 zero-value's + 3 x SD, extrapolated on the standard curve) is expected to be < 5 pg/ml.

## REFERENCES

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E11-06



IL-6

Microtiterstrip ELISA kit

96 coated wells



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