

Table of Contents

| | | |
|-------|--|----|
| 1. | Intended use..... | 2 |
| 2. | Introduction..... | 2 |
| 2.1. | Summary..... | 2 |
| 2.2. | Principle of the method..... | 3 |
| 3. | Reagents provided and reconstitution..... | 4 |
| 4. | Materials required but not provided..... | 4 |
| 5. | Storage Instructions..... | 5 |
| 6. | Specimen collection, processing & storage..... | 5 |
| 7. | Safety & precautions for use..... | 6 |
| 8. | Assay Preparation..... | 7 |
| 8.1. | Assay Design..... | 7 |
| 8.2. | Preparation of Wash Buffer..... | 7 |
| 8.3. | Preparation of Standard Diluent Buffer 1X..... | 7 |
| 8.4. | Preparation of Standard..... | 8 |
| 8.5. | Preparation of Control..... | 8 |
| 8.6. | Preparation of Biotinylated Anti-IL-6..... | 8 |
| 8.7. | Preparation of Streptavidin-HRP..... | 9 |
| 9. | Method..... | 10 |
| 10. | Data Analysis..... | 11 |
| 11. | Assay limitations..... | 11 |
| 12. | Performance Characteristics..... | 12 |
| 12.1. | Sensitivity..... | 12 |
| 12.2. | Specificity..... | 12 |
| 12.3. | Precision..... | 12 |
| 12.4. | Dilution Parallelism..... | 13 |
| 12.5. | Spike Recovery..... | 13 |
| 12.6. | Stability..... | 13 |
| 12.7. | Expected serum values..... | 13 |
| 12.8. | Standard Calibration..... | 13 |
| 13. | Bibliography..... | 14 |
| 14. | Diaclone Human IL-6 ELISA references..... | 14 |
| 15. | Assay Summary..... | 18 |

Human IL-6 ELISA KIT

1. Intended use

The Diaclone Human IL-6 ELISA kit is a solid phase sandwich ELISA for the *in-vitro* qualitative and quantitative determination of IL-6 in supernatants, buffered solutions or serum and plasma samples. This assay will recognise both natural and recombinant human IL-6.

This kit has been configured for research use only. Not suitable for use in therapeutic procedures.

2. Introduction

2.1. Summary

Interleukin-6 (IL-6) is a multi-functional cytokine that regulates immune responses, acute phase reactions and hematopoiesis and may play a central role in host defense mechanisms (13, 31). The gene for human IL-6 has been localized to chromosome 7p21 (1). The genomic sequence has been determined (36). IL-6 is usually not produced constitutively by normal cells, but its expression is readily induced by a variety of cytokines (28), lipopolysaccharide (25) or viral infections (3). The IL-6 gene product is a single chain protein with a molecular mass ranging from 21 to 28 kDa, depending on the cellular source. Extensive posttranslational modifications like N- and O-linked glycosylation (20) as well as phosphorylation (21) seem to account for this heterogeneity. The cDNA for IL-6 predicts a precursor protein of 212 amino acids (10). IL-6 is a pleiotropic cytokine produced by a variety of cells. It acts on a wide range of tissues, exerting growth-induction, growth-inhibition, and differentiation respectively, depending on the nature of the target cells.

IL-6 is involved in

- the induction of B-cell differentiation,
- the induction of acute phase proteins in liver cells,
- growth promotion of myeloma/plasmacytoma/hybridoma cells,
- induction of IL-2 and IL-2 receptor expression,
- proliferation and differentiation of T cells,
- inhibition of cell growth of certain myeloid leukemic cell lines and induction of their differentiation to macrophages,
- enhancement of IL-3-induced multipotential colony cell formation in hematopoietic stem cells and induction of maturation of megakaryocytes as a thrombopoietic factor,
- induction of mesangial cell growth,
- induction of neural differentiation of PC cells (12)
- induction of keratinocyte growth (14).

The abnormal production of IL-6 was first suggested to be related to polyclonal B-cell activation with autoantibody production in patients with cardiac myxoma (9). Since then, IL-6 has been suggested to be involved in the pathogenesis of a variety of diseases. Measurement of IL-6 levels in serum and other body fluids thus provides more detailed insights into various pathological situations. For Example:

Infections:

Body fluids of patients with acute local bacterial or viral infections and serum of patients with gram-negative or positive bacteremia contain elevated levels of biologically active IL-6 (7, 16).

Obstetric Infections:

IL-6 has emerged as a reporter cytokine for intra-amniotic infection (29).

Diseases associated with an altered immune system (polyclonal B-cell abnormalities or autoimmune diseases):

Elevated levels of circulating IL-6 have been detected in patients with cardiac myxoma (11), Castleman's disease (18), rheumatoid arthritis (12), IgM gammopathy and in those with acquired immunodeficiency syndrome (19, 23) as well as alcoholic liver cirrhosis (2, 32).

Proliferative diseases:

Elevated plasma levels of IL-6 are observed in patients with psoriasis (4, 5) and mesangial proliferative glomerulonephritis (15).

Neoplastic Diseases:

Increased systemic levels of IL-6 have been detected in patients with multiple myeloma (22), other B-cell dyscrasias (27), Lennert's T lymphoma, Castleman's disease, renal cell carcinoma (33) and various other solid tumors (17, 30).

Inflammatory responses:

IL-6 is involved in the induction of acute phase proteins and induction of fever (8). Elevated serum levels of IL-6 are also found in patients with severe burns (24, 34), in serum and plasma as a marker for predicting postoperative complications (26), in serum and urine of recipients of kidney transplants before rejection (35), in the serum of septic shock patients (6) and in patients with inflammatory arthritis and traumatic arthritis.

2.2. Principle of the method

A capture Antibody highly specific for IL-6 has been coated to the wells of the microtiter strip plate provided during manufacture. Binding of IL-6 samples and known standards to the capture antibodies and subsequent binding of the Biotinylated anti-IL-6 secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed.

The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing.

A chromogen substrate is added to the wells resulting in the progressive development of a blue coloured complex with the conjugate. The colour development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced coloured complex is directly proportional to the concentration of IL-6 present in the samples and standards.

The absorbance of the colour complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of IL-6 in any sample tested.

3. Reagents provided and reconstitution

| Reagents (Store @ 2-8°C) | Quantity 1x96-well kit Cat no. 950.030.096 | Quantity 2x96-well kit Cat no. 950.030.192 | Reconstitution |
|--|--|--|---|
| Anti-IL-6 Coated Plate | 1 | 2 | Ready to use (96-well strips pre-coated plate) |
| Plastic plate covers | 2 | 4 | n/a |
| IL-6 Standard: 200 pg/ml | 2 | 4 | Reconstitute as directed on the vial (see Assay preparation, section 8) |
| Standard Diluent (Buffer) | 1 (15ml) | 1 (25ml) | 10x concentrate, dilute in distilled water (see Assay preparation, section 8) |
| Standard Diluent Serum | 1 (7 ml) | 2 (7 ml) | Ready to use |
| IL-6 Control | 2 | 4 | Reconstitute as directed on the vial (see Assay preparation, section 8) |
| Biotinylated Anti-IL-6 | 1 (0.4ml) | 2 (0.4ml) | Dilute in Biotinylated Antibody Diluent (see Assay preparation, section 8) |
| Biotinylated Antibody Diluent | 1 (7ml) | 1 (13ml) | Ready to use |
| Streptavidin-HRP | 2 (5µl) | 4 (5µl) | Add 0.5ml of Streptavidin-HRP Diluent prior to use (see Assay preparation, section 8) |
| Streptavidin-HRP Diluent | 1 (12ml) | 1 (23ml) | Ready to use |
| Wash Buffer | 1 (10ml) | 2 (10ml) | 200x concentrate dilute in distilled water (see Assay preparation, section 8) |
| TMB Substrate | 1 (11ml) | 1 (24ml) | Ready to use |
| H ₂ SO ₄ Stop Reagent | 1 (11ml) | 2 (11ml) | Ready to use |

4. Materials required but not provided

- Microtiter plate reader fitted with appropriate filters (450 nm required with optional 620 nm reference filter)
- Microtiter plate washer or wash bottle
- 10, 50, 100, 200 and 1,000µl adjustable single channel micropipettes with disposable tips
- 50-300µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile

5. Storage Instructions

Store kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C). Expiry of the kit and reagents is stated on box front labels. The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

Wash Buffer 1X: Once prepared, store at 2-8°C for up to 1 week.

Standard Diluent Buffer 1X: Once prepared, store at 2-8°C for up to 1 week.

Reconstituted Standard/Control: Once prepared use immediately and do not store.

Diluted Biotinylated Anti-IL-6: Once prepared use immediately and do not store.

Diluted Streptavidin-HRP: Once prepared use immediately and do not store.

6. Specimen collection, processing & storage

Cell culture supernatants, human serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Cell culture supernatants: Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.

Serum: Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

Plasma: EDTA, citrate and heparin plasma can be assayed. Spin samples at 1000 x g for 30 min to remove particulates. Harvest plasma.

Storage: If not analysed shortly after collection, samples should be aliquoted (250-500µl) to avoid repeated freeze-thaw cycles and stored frozen at -70°C. Avoid multiple freeze-thaw cycles of frozen specimens.

Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before use. When possible avoid use of badly haemolysed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

7. Safety & precautions for use

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g. CDC/NIH Health manual : "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with H₂SO₄ and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated as indicated on vials or bottles labels.
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of H₂SO₄ and TMB Substrates solutions, avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB Substrate solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB Substrate solution with metal to prevent colour development. Warning TMB Substrate is toxic avoid direct contact with hands. Dispose off properly.
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- Dispense the TMB Substrate within 15 min of the washing of the microtiter plate.

8. Assay Preparation

Bring all reagents to room temperature before use

8.1. Assay Design

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard, zero and control should be tested **in duplicate**. Remove sufficient microwell strips for testing from the pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8°C storage.

Example plate layout (example shown for a 6 point standard curve)

| | Standards / Controls | | Sample Wells | | | | | | | | | |
|---|----------------------|------|--------------|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 200 | 200 | | | | | | | | | | |
| B | 100 | 100 | | | | | | | | | | |
| C | 50 | 50 | | | | | | | | | | |
| D | 25 | 25 | | | | | | | | | | |
| E | 12.5 | 12.5 | | | | | | | | | | |
| F | 6.25 | 6.25 | | | | | | | | | | |
| G | zero | zero | | | | | | | | | | |
| H | Ctrl | Ctrl | | | | | | | | | | |

All remaining empty wells can be used to test samples in duplicate

8.2. Preparation of Wash Buffer

If crystals have formed in the concentrate Wash Buffer, warm it gently until complete dissolution.

Dilute the (200X) concentrate Wash Buffer 200 fold with distilled water to give a 1X working solution. Pour entire contents (10 ml) of the concentrate Wash Buffer into a clean 2,000 ml graduated cylinder. Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2°-25°C.

8.3. Preparation of Standard Diluent Buffer 1X

If crystals have formed in the concentrate Standard Diluent, warm it gently until complete dissolution.

Dilute the (10X) concentrate Standard Diluent 10 fold with distilled water to give a 1X working solution. Pour entire contents of the concentrate Standard Diluent into a clean appropriate graduated cylinder. Bring to final volume with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2°-25°C. Please see example volumes below:

| Standard Diluent concentrate (ml) | Distilled water (ml) |
|-----------------------------------|----------------------|
| 15 | 135 |
| 25 | 225 |

8.4. Preparation of Standard

Depending on the type of samples you are assaying, the kit may include two Standard Diluents. Because biological fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure, you should reconstitute standard vials with the most appropriate Standard Diluent.

For **serum and plasma** samples : use Standard Diluent - Serum

For **cell culture supernatants** : use Standard Diluent Buffer 1X

Standard vials must be reconstituted with the volume of Standard Diluent shown on the vial immediately prior to use. This reconstitution gives a stock solution of 200 pg/ml of IL-6. Mix the reconstituted standard gently by inversion only. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 200 to 6.25 pg/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 200µl of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 200 pg/ml.
- Add 100µl of Standard Diluent to the remaining standard wells B1 and B2 to F1 and F2.
- Transfer 100µl from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.
- Continue this 1:1 dilution using 100µl from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 200 pg/ml to 6.25 pg/ml.
- Discard 100µl from the final wells of the standard curve (F1 and F2).

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred into the relevant wells.

8.5. Preparation of Control

Freeze-dried control vials should also be reconstituted with the most appropriate Standard Diluent to your samples.

For **serum and plasma** samples : use Standard Diluent - Serum

For **cells culture supernatants** : use Standard Diluent Buffer 1X

The supplied Control must be reconstituted with the volume of Standard Diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume, will give a solution at the concentration stated on the vial. Do not store after use.

8.6. Preparation of Biotinylated Anti-IL-6

It is recommended this reagent is prepared immediately before use. Dilute the Biotinylated Anti-IL-6 with the Biotinylated Antibody Diluent in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

| Number of wells required | Biotinylated Antibody (µl) | Biotinylated Antibody Diluent (µl) |
|--------------------------|----------------------------|------------------------------------|
| 16 | 40 | 1060 |
| 24 | 60 | 1590 |
| 32 | 80 | 2120 |
| 48 | 120 | 3180 |
| 96 | 240 | 6360 |

8.7. Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.

Dilute the 5 μ l vial with 0.5ml of Streptavidin-HRP Diluent **immediately before use**. Do not keep this diluted vial for future experiments. Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial. Please see example volumes below:

| Number of wells required | Streptavidin-HRP (μ l) | Streptavidin-HRP Diluent (ml) |
|--------------------------|-----------------------------|-------------------------------|
| 16 | 30 | 2 |
| 24 | 45 | 3 |
| 32 | 60 | 4 |
| 48 | 75 | 5 |
| 96 | 150 | 10 |

9. Method

We strongly recommend that every vial is mixed thoroughly without foaming prior to use.

Prepare all reagents as shown in section 8.

Note: final preparation of **Biotinylated Antibody** (section 8.6) and **Streptavidin-HRP** (section 8.7) should occur immediately before use.

| Assay Step | | Details |
|--|------------|---|
| 1. | Addition | Prepare standard curve as shown in section 8.4 above and add in duplicate to appropriate wells |
| 2. | Addition | Add 100µl of each Sample, Control and zero (appropriate Standard Diluent) in duplicate to appropriate number of wells |
| 3. | Addition | Add 50µl of diluted Biotinylated Anti-IL-6 to all wells |
| 4. | Incubation | Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 1 hour |
| 5. | Wash | Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.3 ml of 1x Wash Buffer into each well c) Aspirate the contents of each well d) Repeat step b and c another two times |
| 6. | Addition | Add 100µl of diluted Streptavidin-HRP solution into all wells |
| 7. | Incubation | Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 30 min |
| 8. | Wash | Repeat wash step 5. |
| 9. | Addition | Add 100µl of ready-to-use TMB Substrate into all wells |
| 10. | Incubation | Incubate in the dark for 12-15 minutes* at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil. |
| 11. | Addition | Add 100µl of H₂SO₄ Stop Reagent into all wells |
| <p>Read the absorbance value of each well (immediately after step 11.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).</p> | | |

** Incubation time of the TMB substrate is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.*

10. Data Analysis

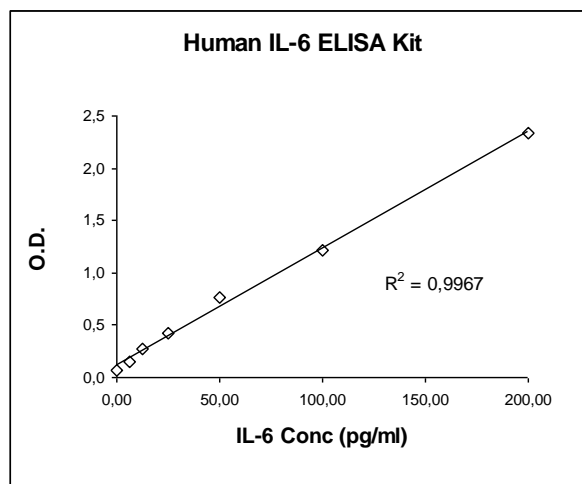
Calculate the average absorbance values for each set of duplicate standards, control and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding IL-6 standard concentration on the horizontal axis.

The amount of IL-6 in each sample is determined by extrapolating OD values against IL-6 standard concentrations using the standard curve.

Example IL-6 Standard curve

| Standard | IL-6 Conc (pg/ml) | OD (450nm) mean | CV (%) |
|----------|-------------------|-----------------|--------|
| 1 | 200 | 2.34 | 11.4 |
| 2 | 100 | 1.21 | 4.5 |
| 3 | 50 | 0.76 | 4.0 |
| 4 | 25 | 0.43 | 0.0 |
| 5 | 12.5 | 0.27 | 2.6 |
| 6 | 6.25 | 0.15 | 4.6 |
| zero | 0 | 0.07 | - |



Note: curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

11. Assay limitations

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard Diluent Buffer or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

12. Performance Characteristics

12.1. Sensitivity

The sensitivity or minimum detectable dose of IL-6 using this Diaclone Human IL-6 ELISA kit was found to be **2pg/ml**. This was determined by adding 3 standard deviations to the mean OD obtained when the zero standard was assayed 40 times.

12.2. Specificity

The assay recognizes both natural and recombinant human IL-6. To define the specificity of this ELISA several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested: IL-1 α , IL-1 β , IL-10, IL-12, IFN γ , IL-4, TNF α , IL-8 and IL-13.

12.3. Precision

Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates (3 duplicates) of samples containing different concentrations of IL-6: 3 in human pooled Serum and 2 in Supernatant. 2 standard curve were run on each plate. Data below show the mean IL-6 concentration and the coefficient of variation for each sample.

The calculated overall coefficient of variation was 3.6%.

| Session | Sample | Mean IL-6 pg/ml | SD | CV% |
|-----------|----------|-----------------|------|-----|
| Session 1 | Sample 1 | 213.3 | 6.0 | 2.8 |
| | Sample 2 | 85.0 | 2.9 | 3.4 |
| | Sample 3 | 61.5 | 2.6 | 4.3 |
| | Sample 4 | 101.3 | 1.4 | 1.4 |
| | Sample 5 | 50.7 | 0.9 | 1.7 |
| Session 2 | Sample 1 | 193.3 | 3.9 | 2.0 |
| | Sample 2 | 72.8 | 4.6 | 6.3 |
| | Sample 3 | 60.9 | 3.0 | 4.9 |
| | Sample 4 | 101.5 | 4.7 | 4.6 |
| | Sample 5 | 52.6 | 1.7 | 3.2 |
| Session 3 | Sample 1 | 176.4 | 16.5 | 9.4 |
| | Sample 2 | 69.9 | 1.4 | 2.0 |
| | Sample 3 | 58.5 | 2.8 | 4.8 |
| | Sample 4 | 105.9 | 0.9 | 0.9 |
| | Sample 5 | 51.0 | 0.8 | 1.6 |

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by two technicians. Each assay was carried out with 6 replicates (3 duplicates) of samples containing different concentrations of IL-6: 3 in human pooled Serum and 2 in Supernatant. 2 standard curve were run on each plate. Data below show the mean IL-6 concentration and the coefficient of variation for each sample.

The calculated overall coefficient of variation was 7.7%.

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Mean IL-6 pg/ml | 200 | 77 | 63 | 103 | 53 |
| SD | 16 | 8 | 6 | 6 | 3 |
| CV% | 8.1 | 10.1 | 8.8 | 6.0 | 5.5 |

12.4. Dilution Parallelism

In two independent experiments two spiked human serum samples with different levels of IL-6 were analysed at different serial two fold dilutions (1:2 to 1:8) with two replicates each.

Recoveries ranged from 78 to 116% with an overall **mean recovery of 96%**.

12.5. Spike Recovery

The spike recovery was evaluated by spiking 3 concentrations of IL-6 in human Serum and culture medium in 3 separate experiments.

Recoveries ranged from 93 to 115% with an overall **mean recovery of 103%**.

12.6. Stability

Storage Stability

Aliquots of spiked serum and spiked medium were stored at -20°C , $+2-8^{\circ}\text{C}$, room temperature (RT) and at 37°C and the IL-6 level determined after 24h. There was no significant loss of IL-6 reactivity during storage at $+2-8^{\circ}\text{C}$, RT and 37°C .

Freeze-thaw Stability

Aliquots of spiked serum and spiked medium were stored frozen at -20°C and thawed up to 5 times and the IL-6 level was determined. There was no significant loss of IL-6 reactivity after 5 cycles of freezing and thawing.

12.7. Expected serum values

A panel of 20 human sera was tested for IL-6. All were below the detection level of 2pg/ml.

12.8. Standard Calibration

This immunoassay is calibrated against the International Reference Standard NIBSC 89/548. NIBSC 89/548 is quantitated in International Units (IU) and equivalence in pg/ml is indicated.

It has been calculated that 1IU NIBSC (approximately 10 pg) corresponding to 11 pg Diaclone IL-6.

13. Bibliography

1. Bowcock AM and al. Genomics. 1988 Jul;3(1):8-16.
2. Byl B. and al. Gastroenterology. 1993 May;104(5):1492-7.
3. Cayphas S. and al. J Immunol. 1987 Nov 1;139(9):2965-9.
4. Elder JT and al. Arch Dermatol Res. 1992;284(6):324-32.
5. Grossman RM and al. Proc Natl Acad Sci U S A. 1989 Aug;86(16):6367-71.
6. Hack CE and al. Blood. 1989 Oct;74(5):1704-10.
7. Helfgott DC and al. J Immunol. 1989 Feb 1;142(3):948-53.
8. Helle M and al. Eur J Immunol. 1988 Jun;18(6):957-9.
9. Hirano T and al. Proc Natl Acad Sci U S A. 1985 Aug;82(16):5490-4.
10. Hirano T and al. Nature. 1986 Nov 6-12;324(6092):73-6.
11. Hirano T and al. Proc Natl Acad Sci U S A. 1987 Jan;84(1):228-31.
12. Hirano T and al. Eur J Immunol. 1988 Nov;18(11):1797-801.
13. Hirano T and al. Immunol Today. 1990 Dec;11(12):443-9.
14. Horii Y and al. Kidney Int Suppl. 1993 Jan;39:S71-5.
15. Houssiau FA and al. Clin Exp Immunol. 1988 Feb;71(2):320-3.
16. Kishimoto T. Blood. 1989 Jul;74(1):1-10.
17. Kishimoto T. And al. Annu Rev Immunol. 1988;6:485-512.
18. Martínez-Maza O Res Immunol. 1992 Sep;143(7):764-9.
19. May LT and al. J Biol Chem. 1988 Jun 5;263(16):7760-6.
20. May LT and al. Biochem Biophys Res Commun. 1988 May 16;152(3):1144-50.
21. Merico F and al. Clin Exp Immunol. 1993 Apr;92(1):27-31.
22. Nakajima K and al. J Immunol. 1989 Jan 15;142(2):531-6.
23. Nijsten MW and al. Lancet. 1987 Oct 17;2(8564):921.
24. Nordan R and al. Science. 1986 Aug 1;233(4763):566-9.
25. Oka Y and al. Cytokine. 1992 Jul;4(4):298-304.
26. Pettersson T and al. J Intern Med. 1992 Nov;232(5):439-42.
27. Ray A and al. Ann N Y Acad Sci. 1989;557:353-61; discussion 361-2.
28. Santhanam U and al. Cytokine. 1991 Mar;3(2):155-63.
29. Seguchi T and al. J Urol. 1992 Sep;148(3):791-4.
30. Sehgal PB and al. J Exp Med. 1988 Jun 1;167(6):1951-6.
31. Sheron N and al. Clin Exp Immunol. 1991 Jun;84(3):449-53.
32. Tsukamoto T and al. J Urol. 1992 Dec;148(6):1778-81; discussion 1781-2.
33. Ueyama M and al. J Lab Clin Med. 1992 Nov;120(5):693-8.

14. Diaclone Human IL-6 ELISA references

1. Agahi, A. et al., Front Neurol. 2018 Aug 15;9:662
2. Akbari, F. et al., Iran J Neurol.,2016; 15(2): 75-9
3. Akbarpour, M., Biol Sport,2013; 30(1): 21-7
4. Akram Kooshki, A. et al.,Glob J Health Sci.,2015; 7(7 Spec No): 1-5
5. Arora,V. et al.,J Anaesthesiol Clin Pharmacol. 2017 Jan-Mar;33(1):57-63
6. Ashraf, H. et al., Indian J Endocrinol Metab. 2018 Nov-Dec;22(6):751-756.
7. Auf, G. et al., PNAS,2010;0914072107
8. Azadbakht, L. et al., Diabetes Care,2007;30(4):967-73
9. Badoual, C. et al., Cancer Res.,2008; 68(10): 3907-3914.
10. Balabanian K. et al., Am. J. Respir. Crit. Care Med., 2002; 165(10): 1419 - 1425
11. Barber, M. D. et al., Br J Cancer,2004 ; 90(6): 1129-32
12. Biswas, S. et al.,Korean J Intern Med.,2010 ; 25(1): 44-50
13. Bonnans, C. et al., Am J Pathol., 2006; 168 (4) :1064-72.
14. Bozic, M. et al.,PLoS One,2015;10(8): e0136863
15. Burger, R. et al., Hematol J.,2001; 2(1): 42-53.
16. Calabro, M. L. et al., Blood,2009; 113(19): 4525-4533.
17. Capo, X. et al., Nutrients,2016; 8(10).
18. Capo, X. et al., Oxid Med Cell Longev., 2015: 187849

19. Chabbert-de Ponnat, I. et al., *Int Immunol.*,2005; 17(4): 439-47.
20. Chang K-T. et al., *Am J Physiol Lung Cell Mol Physiol*, 2005; 289(3): L438-45
21. Chang, K. T. et al., *Am J Physiol Lung Cell Mol Physiol.*,2005; 289(3): L446-53.
22. Chavushyan, A. et al., *Schizophr Res Treatment*, 2013: 125264
23. Chen, Y. C. et al., *Mediators Inflamm.*, 2010: 573594
24. Coles, J. G. et al., *J Thorac Cardiovasc Surg.*,2005;129(5): 1128-36.
25. Corbi P. et al., *Eur J Cardiothorac Surg.*, 2000; 18(1): 98 - 103
26. Corvaisier, M. et al., *PLoS Biol.*,2012;10(9): e1001395
27. Cot, M. et al.,*PLoS One*,2011; 6(10): e26316
28. Cunin, P. et al., *J. Immunol.*,2011;186(7): 4175-4182.
29. Cutolo M. et al., *Ann. Rheum. Dis*, 2005; 64(2): 212 - 216
30. Cutolo M. et al., *Ann. Rheum. Dis*, 2005; 65(6): 728-35
31. Cutolo, M. et al., *Arthritis Res Ther.*,2009;11(6): R176
32. de Gonzalo-Calvo, D. et al., *Age (Dordr)*,2011; 34(3): 761-71
33. de Haan, J. J. et al.,*Crit Care*,2009; 13(3): R86
34. Dineshkumar, T. et al.,*Asian Pac J Cancer Prev*. 2016 Nov 1;17(11):4899-4906.
35. Dore, P. et al., *Clin Exp Immunol.*,1997; 107(1): 182-8
36. Ehlermann, P. et al.,*Cardiovasc Diabetol.*,2006; 5: 6
37. Eilertsen, G. O. et al., *Lupus*,2011 ; 20(6): 607-613.
38. Faam, B. et al., *Iran J Basic Med Sci.*,2014;17(8): 577-82
39. Ferrer, M.D. et al., *Nutrients*. 2018 Nov 16;10(11). pii: E1780.
40. Forsbach, A. et al., *J. Immunol.*,2008;180(6): 3729-3738.
41. Fu, J. F. et al.,*World J Gastroenterol.*,2009; 15(8):912-8
42. Garcia-Posadas, L. et al., *Invest. Ophthalmol. Vis. Sci.*, 2013 ; 54(10): 7143-7152
43. Garcia-Posadas, L. et al., *PLoS One*,2017; 12(3): e0171099
44. Gehrke, T. et al., *Oncol Lett.*,2016; 12(5): 3549-3554
45. Giamarellou-Bourboulis, E. J. et al., *Crit Care*,2006; 10(3): R76
46. Giamarellou-Bourboulis, E. J. et al., *PLoS One*,2009; 4(12): e8393
47. Gironella, M. et al., *Gut* ,2005; 54(9): 1244-53.
48. Gombocz, K. et al., *Crit Care*,2007; 11(4): R87
49. Gomez-Tortosa, E. et al., *Arch Neurol.*, 2003; 60(9): 1218-22
50. Rodríguez González-Herrero, M.E. et al.,*Clin Ophthalmol*. 2018 May 29;12:1011-1020.
51. Gougelet, A. et al., *PLoS One*,2009; 4(11): e8026
52. Goyal, S. et al.,*Ind Psychiatry J*. 2017 Jul-Dec;26(2): 201-206.
53. Guerrero, A. et al., *Aging Cell*,2015; 14(2): 274-83
54. Gulati, S. et al.,*Am J Reprod Immunol*, 2012; 67(3): 235-40
55. Guldiken, B. et al., *Angiology*,2008; 59(2): 224-229.
56. Gupta, A. et al., *Human and Experimental Toxicology*,2011;30(10): 1445-1453
57. Hjuler Nielsen, M. et al., *PLoS One*,2015;10(4): e0121516
58. Iida, K. et al., *Oncol Lett*. 2019 Apr;17(4):4004-4010.
59. Jengo, G. et al., *Blood*, 1999; 94(2): 701-12
60. Jouan, J. et al., *J. Thorac. Cardiovasc. Surg.*, 2012;144(2):467-4732
61. Kanakdande, V. et al.,*Contemp Clin Dent.*,2015; 6(3): 348-57
62. Kashyap, B. et al., *J Trop Pediatr.*,2011:fmr093
63. Kerr J. et al., *J. Gen. Virol.*, 2001; 82(Pt 12): 3011-3019
64. Koussoulas, V. et al.,*World J Gastroenterol.*,2006; 12(41): 6711-4
65. Krasimirova, E. et al., *World J Exp Med.*, 2017 Aug 20;7(3):84-96.
66. Kruger, K. et al., *J Appl Physiol.*,2011; 110(5): 1226-1232
67. Kube D. et al., *Blood*, 2001; 98(3): 762 - 770
68. Kulms D. et al., *J. Biol. Chem.*, 2000; 275(20): 15060 - 15066
69. Kumpf, O. et al. ,*Crit Care*,2010; 14(3): R103.
70. Kurowski, M. et al.,*Arch Med Sci*. 2018 Jan;14(1):60-68
71. Kwon K.Y. et al., *J. Korean Med. Sci.*, 2001; 16(6): 774 - 780
72. Kyriakopoulou, M. et al., *Mediators Inflamm.*, 2008: 450196.
73. Lafuente, M. et al.,*Retina*. 2019 Jun;39(6):1083-1090.
74. Le Meur Y. et al., *Nephrol. Dial. Transplant.*, 1999; 14(10) : 2420 - 2426
75. Lee, H. W. et al., *Ann Dermatol.*,2013 ; 25(2): 173-80
76. Leifsdottir, KJ. et al., *Neuroinflammation*. 2018 Aug 8;15(1):223.
77. Lekkou A. et al., *Clin. Diagn. Lab. Immunol.*, 2004; 11(1):161 - 167

78. Lewis, S. et al., *Respir Res.*,2009 ; 10: 44
79. Liao, B. et al., *Emerg Microbes Infect.*,2015; 4(4): e24
80. Lieblein, J. C. et al.,*BMC Cancer*,2008; 8: 302
81. Loo, W. T. et al., *J Transl Med.*,2012; 10 Suppl 1: S8
82. Lotrich, F. E. et al., *Brain Behav Immun.* ,2013;31: 48-53.
83. Lu, C. et al., *Lupus*, 2015;24(1):18-24
84. Madempudi, R.S. et al.,*Sci Rep.* 2019 Aug 21;9(1):12210.
85. Madempudi, R.S. et al.,*Sci Rep.* 2019 Aug 21;9(1):12210.
86. Mahalle, N. et al., *Ann Med Health Sci Res.*, 2014; 4(5): 706-12
87. Mahalle, N. et al., *Indian J Endocrinol Metab.*2013; 17(5): 844-50
88. Mahalle, N. et al., *Indian J Endocrinol Metab.*2014 ; 18(1): 48-55
89. Mallick, B. et al., *JGH Open.* 2019 Mar 12;3(4): 295-301.
90. Mateen,S. et al.,*PLoS One.* 2017 Jun 8;12(6):e0178879
91. Matsui, S. et al.,*Inflamm Res.* 2018 Dec;67(11-12):965-973.
92. Mochel, F. et al., *PLoS One*,2007; 2(7): e647.
93. Mohamed, H. S. and M. M. Meguid, *Saudi J Anaesth.*,2017;11(1): 9-14.
94. Mokhtare, M. et al.,*Middle East J Dig Dis.* 2017 Oct;9(4):228-234.
95. Montes, C. L. et al.,*Cancer Res.*,(2008; 68(3): 870-879.
96. Moreaux, J. et al., *Blood*,2011; 117(4): 1280-1290
97. Mori, T. et al., *Exp Anim.*,2016; 65(4): 455-463.
98. Musleh, G. S. et al.,*Eur. J. Cardiothorac. Surg.*,2009; 35(3): 511-514.
99. Nagao, Y. et al., *Malar J.*,2008; 7: 113.
100. Nebor, D. et al., *Haematologica*,2011;96(11):1589-1594
101. Netam, R. et al., *Indian J Med Res.*,2015; 141(6): 775-82
102. Onambele-Pearson, G. L. and S. J. Pearson, *Age (Dordr)*,2012;34(2): 427-38
103. Onambele-Pearson, G. L. et al., *Age (Dordr)*,2010; 32(2): 139-53
104. Onambele-Pearson, G. L. et al.,*Age (Dordr)*,2010; 32(2): 125-38
105. Pac-Kozuchowska, E. et al., *Med Sci Monit.*,2016;22: 1534-9.
106. Paduch, R. and A. Wozniak, *J Ophthalmic Vis Res.*,2015; 10(3): 229-37
107. Paduch, R., et al., *Balkan Med J.*,2014 ; 31(1): 29-36
108. Panes, J. et al., *Am J Physiol Gastrointest Liver Physiol.*,2007; 293: G739 – G748
109. Pasi, F. et al., *Anticancer Res.*,2010; 30(7): 2769-2772
110. Pelekanou, A. et al.,*Crit Care*,2009; 13(6): R172
111. Peng, L. S. et al.,*World J Emerg Med.*,2015 ; 6(2): 123-30
112. Perret D. et al., *J. Biol. Chem.*, 2004; 279(42): 43961 - 43970
113. Perry, M. G. et al., *Rheumatology(Oxford)*,2006;45(2): 229-30.
114. Pitta, M. G. et al., *J Clin Invest.*,2009; 119(8): 2379-87
115. Popa, S.L. et al.,*Medicine (Baltimore)*. 2018 Dec;97(49):e13562
116. Prakash, S. et al., *Clin Orthop Relat Res.*,2013; 471(7): 2340-6
117. Prather, A. A. et al.,*Brain Behav Immun.*, 2009;23(8): 1109-16
118. Puthier, D. et al., *Blood*,1996; 88(12): 4659-66
119. Rai, G. et al.,*Ann Lab Med.*, 2018 Mar;38(2):125-131
120. Rajappa, M. et al., *Angiology*,2009; 60(4): 419-426.
121. Rana, S. V. et al., *J Crohns Colitis*,2014 ; 8(8): 859-865
122. Rasool, R. et al., *Asia Pac Allergy*,2014 ; 4(4): 206-11
123. Robertson, M. D. et al., *J. Clin. Endocrinol. Metab.*,2012; 97(9): 3326-3332.
124. Rodríguez-Luna, A. et al., *Mar Drugs*. 2018 Oct 10;16(10). pii: E378.
125. Rotter V. et al., *J. Biol. Chem.*, 2003; 278(46): 45777 - 45784
126. Routsis, C. et al.,*Clin Exp Immunol.*,2005;142(1): 62-7.
127. Rybka, J. et al.,*Clin Exp Med.*,2015; 16(4): 493-502.
128. Sell, H. et al., *Am J Physiol Endocrinol Metab.*, 2008;294:E1070-E1077
129. Shadman, Z. et al., *J Diabetes Metab Disord.*,2013; 12(1): 42
130. Shete, S.U. et al.,*J Educ Health Promot.* 2017 Aug 9;6:76
131. Shetty, P. et al., *Annals of Clinical Biochemistry*,2016: 0004563216665867
132. Shpacovitch V.M. et al., *J. Leukoc. Biol.*, 2004; 76(2): 388 - 398
133. Sopasakis V. et al., *Obes. Res.*, 2004; 12(3): 454 - 460
134. Soriano-Izquierdo A. et al., *J. Leukoc. Biol.*, 2004; 75(2): 214 - 223
135. Souza, L. S. et al., *J Rheumatol.*,2008; 35(11): 2265-2271.
136. Stebbing J. et al., *Ann. Onc.*, 2003; 14(11): 1660 - 1666

137. Stebbing, J. et al., Clin Exp Immunol.,2004; 138(2): 312-6.
138. Stefansson, B. V. et al., Nephron Extra,2012; 2(1): 55-65.
139. Sun, Y. et al., J. Immunol.,2008; 180(6): 4173-4181.
140. Sun, Z. et al.,Cent Eur J Immunol.,2014; 39(2): 216-22
141. Sundaresh, A. et al.,Heliyon. 2019 Jan 7;5(1):e01124.
142. Sureda, A. et al., PLoS One,2016; 11(9): e0163371.
143. Suzuki, N. et al., Tob Induc Dis.,2016; 14: 20.
144. Szot, K. et al., Environ Geochem Health. 2019 Jun;41(3):1577-1582.
145. Szumowska, A. et al., Clinical and Applied Thrombosis/Hemostasis,2016; 22(7): 679-684
146. Tabibi, H. et al., Perit. Dial. Int.,2016; 36(2): 140-145
147. Tang, Y. et al., Am J Trop Med Hyg.,2008; 79(2): 154-158.
148. Tang, Y. et al.,PLoS One,2010; 5(12): e15631
149. Tayal, D. et al.,Indian J Clin Biochem.,2014; 29(2): 139-44
150. Tolahunase, M. et al., Oxid Med Cell Longev. 2017;2017:2784153
151. Tolahunase, M. et al., Oxid Med Cell Longev.,2017; 2017: 7928981.
152. Toutirais, O. et al.,Clin Exp Immunol.,2007 ; 149(2): 372-7
153. Triantafilou K. et al., J. Cell Sci., 2001; 114(Pt 13): 2535 - 2545
154. Trynieszewski, W. et al., Acta Endocrinol (Buchar). 2018 Oct-Dec;14(4):439-446.
155. Tsaganos, T. et al.,BMC Infect Dis.,2006; 6: 142.
156. Türkoğlu, R. et al.,Med Princ Pract. 2014;23(3):239-45.
157. Tuttolomondo, A. et al., Cardiovasc Diabetol.,2010; 9: 50
158. Tuttolomondo, A. et al.,PLoS One,2016; 11(12): e0165443
159. Tyagi,A. et al.,Indian J Crit Care Med. 2019 Feb; 23(2): 89–94
160. Unal,D. et al.,Asia Pac Allergy, 2017;7:74-81
161. Valdor, R. et al.,Oncotarget. 2017 Aug 2;8(40):68614-68626.
162. van Setten, P. A. et al., Blood,1996; 88(1): 174-83.
163. Verghese, B. et al., Indian J Clin Biochem.,2011 ; 26(4): 373-7
164. Versaci, F. et al., Mayo Clin Proc.,2012; 87(1): 50-8
165. Viridis, A., et al.,Eur. Heart J.,2015: ehv365
166. Vita N. et al., J. Immunol., 1997; 158(7): 3457 - 3462
167. Vollmer J. et al., Antimicrob. Agents Chemother., 2004; 48(6): 2314 - 2317
168. Vollmer J. et al., J Leukoc Biol., 2004;76(3) : 585 - 593
169. Vollmer, J. et al.,Immunology,2004; 113(2): 212-23.
170. Walavalkar, V. et al., Eur J Cardiothorac Surg.,2016; 49(5): 1403-1410
171. Williamson, P. A. et al., Eur. Respir. J.,2011; 37(1): 206-209
172. Wong C.K. et al., Am. J. Respir. Cell Mol. Biol., 2005; 33(2): 186 - 194
173. Wrenger, S. et al., J. Leukoc. Biol.,2006; 80(3): 621-629.
174. Yadav, U. C. et al., Chem Biol Interact.,2011; 191(1-3): 339-45
175. Yadav, U. C. et al., PLoS One,2009; 4(8): e6535
176. Yadav, U. C. S. et al., J. Immunol.,2009; 183(7): 4723-4732.
177. Zhang, J. et al.,Tanaffos. 2017 Jun;16(4):260-269.
178. Zhang, Y. et al., Lupus, 2011; 20: 1172 - 1181

15. Assay Summary

Total procedure length: 1h45min

**Add 100µl of Samples, Control and diluted Standards
and 50µl diluted Biotinylated Antibody**



Incubate 1 hour at room temperature



Wash three times



Add 100µl of diluted Streptavidin-HRP



Incubate 30 min at room temperature



Wash three times



**Add 100µl of TMB Substrate
Protect from light. Let the color develop for 12-15 min.**



Add 100µl of Stop Reagent



Read Absorbance at 450 nm

Products Manufactured and Distributed by:

Diaclone SAS
6 Rue Dr Jean-François-Xavier Girod
25000 Besançon
France
Tel +33 (0)3 81 41 38 38
Email: techsupport@medixbiochemica.com