Human IL6 ELISA Kit

Human IL6 ELISA Kit is an enzyme immunoassay kit for the quantification of IL6 in human serum.

Catalog number: ARG83085

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. In humans, it is encoded by the IL6 gene.

In addition, osteoblasts secrete IL-6 to stimulate osteoclast formation. Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine. IL-6's role as an anti-inflammatory myokine is mediated through its inhibitory effects on TNF-alpha and IL-1 and its activation of IL-1ra and IL-10.

There is some early evidence that IL-6 can be used as an inflammatory marker for severe COVID-19 infection with poor prognosis, in the context of the wider coronavirus pandemic.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Human IL6 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IL6 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Human IL6 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Human IL6 bound in the initial step. The color development

is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ± 2nm. The concentration of Human IL6 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C
Standard A-F (0, 23.3, 68, 201, 633, 2560 pg/ml)	6 vials	4°C
Control 1 and 2	2 vials	4°C
Antibody Conjugate	11 ml	4°C
Sample Diluent	2 vials	4°C
Assay Buffer	11 ml	4°C
200X Wash Buffer	10 ml	4°C
TMB Substrate	25 ml (Ready-to-use)	4°C
Stop Solution	12 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-650 nm as reference wavelength)
- Microplate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Store the unopened reagents at 2-8°C until expiration date. Once opened the reagents are stable for 1 month when stored at 2-8°C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at-20 or-70°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 200X Wash buffer into distilled water to yield 1X Wash buffer. Storage: up to 1 months at 2-8°C.
- Standards and Control: Reconstitute the standard and control with 1 ml distilled water. Keep the buffer in the vail for at least 15 min at RT to make sure the standard is dissolved completely before making serial dilutions
- Sample Diluent:

ASSAY PROCEDURE

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
1. Add 50 µl Assay Buffer into all wells.
2. Add 100 µl extracted standards, controls, and samples into appropriate wells.
3. Incubate 60 mins at RT on a microplate shaker (700rpm).
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of

liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add **100 µl** of **Antibody Conjugate** and **50 µl Sample Diluent** into wells.
6. Incubate for **60 mins** at RT on a microplate shaker (700rpm).
7. Aspirate each well and **wash** as step 4.
8. Add **200 µl** of **TMB substrate solution** into each well. Incubate for **10-20 mins** at RT with shaking (700rpm) in dark.
9. Add **100 µl** of **Stop Solution** to each well and shake lightly to ensure homogeneous mixing.
10. Read the OD with a microplate reader at **450nm** (with a reference wavelength between 620nm and 650nm) within 30 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

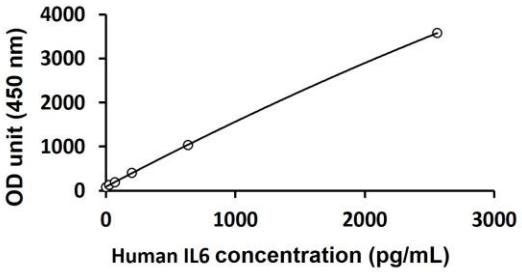
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

11.5 pg/ml

Assay Range

23.3 – 2560 pg/ml

Specificity

Not reacts with IL1 alpha, IL1 beta, IL2, IL3, IL4, IL7, IL8, IL10, GMCSF, IFN alpha, IFN gamma, LIF, MIP-1 alpha, MIP 1beta, MCP1, OSM, RANTES, TGF beta, TNF alpha and TNF beta.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.3% and CV value of inter-assay precision was 4.9%.