Human IL6R alpha ELISA Kit

Enzyme Immunoassay for the quantification of Human IL6R alpha in serum, plasma, cell culture supernatants

Catalog number: ARG81261

Package: 96wells

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INTRODUCTION

IL6R alpha is a subunit of the interleukin 6 (IL6) receptor complex. Interleukin 6 is a potent pleiotropic cytokine that regulates cell growth and differentiation and plays an important role in the immune response. The IL6 receptor is a protein complex consisting of this protein and interleukin 6 signal transducer (IL6ST/GP130/IL6-beta), a receptor subunit also shared by many other cytokines. Dysregulated production of IL6 and this receptor are implicated in the pathogenesis of many diseases, such as multiple myeloma, autoimmune diseases and prostate cancer. Alternatively spliced transcript variants encoding distinct isoforms have been reported. A pseudogene of this gene is found on chromosome 9.[provided by RefSeq, May 2011]

IL6R alpha is part of the receptor for interleukin 6. Binds to IL6 with low affinity, but does not transduce a signal. Signal activation necessitate an association with IL6ST. Activation may lead to the regulation of the immune response, acute-phase reactions and hematopoiesis.

Low concentration of a soluble form of IL6 receptor acts as an agonist of IL6 activity. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IL6R alpha has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IL6R alpha present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for IL6R alpha 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 IL6R alpha 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 IL6R alpha 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. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 X 1 ng/vial	4°C
Standard/Sample diluent buffer	2 X 16 ml	4°C
Antibody conjugate concentrate	2 vials (60 µl)	4°C
Antibody diluent buffer	16 ml	4°C
HRP-Streptavidin concentrate	2 vials (60 µl)	4°C (Protect from light)
HRP-Streptavidin diluent buffer	16 ml	4°C
20X Wash buffer	25 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- 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.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 15-20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.
- The TMB Color developing agent should be colorless and transparent before using.

- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Avoid using reagents from different batches.
- 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.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Note: a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 25 ml of 20X Wash buffer + 475 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- 1X Antibody conjugate: 5-10 minutes before use (freshly prepared is recommended), dilute 100X antibody conjugate concentrate into Antibody diluent buffer with Antibody diluent buffer to yield 1X detection antibody solution.
- 1X HRP-Streptavidin Solution: 5-10 minutes before use (freshly prepared is recommended), dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer.
- Sample: If the initial assay found samples contain IL6R alpha higher than the highest standard, the samples can be diluted with Standard/Sample diluent buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  Note: For normal human serum/plasma samples, they can start with a 1:50-1:100 dilution with Standard/Sample diluent buffer before assay.

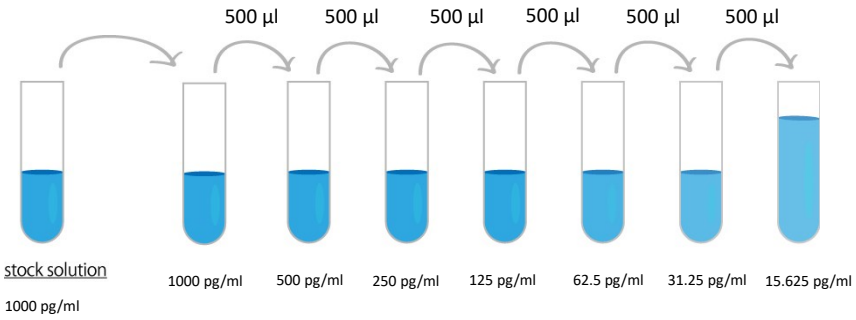
  Example:

  a) Dilution 1:10: 10 µl of sample + 90 µl of Standard/Sample diluent buffer (mix thoroughly).
  b) Dilution 1:50: 20 µl of 1:10 diluted sample from a) + 80 µl of Standard/Sample diluent buffer (mix thoroughly)
  c) Dilution 1:100: 10 µl of 1:10 diluted sample from a) + 90 µl of Standard/Sample diluent buffer (mix thoroughly).

d) For duplicates, make sure to make minimal 250 µl of every sample to be able to transfer 100 µl to the coated plate.

(It is recommended to do pre-test to determine the suitable dilution factor).

- Standards: Reconstitute the standard with 1 ml Standard/Sample diluent buffer to yield a stock concentration of 1000 pg/ml. Allow the stock standard to sit for at least 15 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. Aliquot and store the reconstituted standard for up to 1 month at -20 °C. Avoid repeated freeze-thaw cycles. The Standard/Sample diluent buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.625 pg/ml. Diluted standard shall not be reused.



Dilute IL6R alpha standard as according to the table below:

Standard	IL6R alpha Conc.	µl of Standard/Sample diluent	µl of standard
S7	1000 pg/ml	0	1000 (1000 pg/ml Stock)
S6	500 pg/ml	500	500 (S7)
S5	250 pg/ml	500	500 (S6)
S4	125 pg/ml	500	500 (S5)
S3	62.5 pg/ml	500	500 (S4)
S2	31.25 pg/ml	500	500 (S3)
S1	15.625 pg/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) at least 10-20 min before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. It can be store at 2-8°C for up to 1 month.
2. Add 100 µl of standards, samples and zero controls (Standard/Sample diluent buffer) into wells. Incubate for 1.5 h at 37 °C.
3. Aspirate each well and wash, repeating the process three times for a total four washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the 1X Wash Buffer in the wells for 15-30 sec before remove. 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.

4. Add 100 µl 1X Antibody conjugate into each well. Cover wells and incubate for 1 hour at 37 °C.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of 1X HRP-Streptavidin solution to each well. Cover wells and incubate for 30 minutes at 37 °C.
7. Aspirate each well and wash as step 3.
8. Add 100 µl of TMB Reagent to each well. Incubate for 10-20 minutes at 37°C in dark. (Note: The incubation time is for reference, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated standard solutions; the other wells show no obvious color).
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing
10. Read the OD with a microplate reader at 450nm immediately. (optional: read at 610-650 nm as the reference wave length) It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

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

2. Using 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.

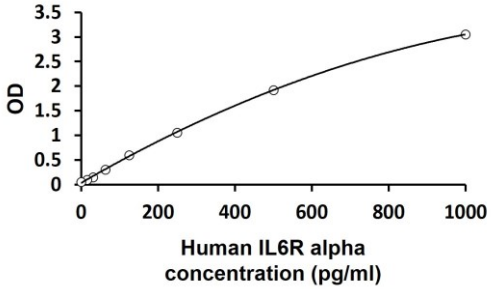
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

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

The minimum detectable dose (MDD) of Human IL6R alpha ranged from 15.6-1000 pg/ml. The mean MDD was 7 pg/ml.

Specificity

This assay recognizes natural and recombinant Human IL6R alpha. No significant cross-reactivity or interference with the factors below was observed:

50 ng/ ml of recombinant proteins:

Human: IL1 ra, IL1 alpha, IL1 beta, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11

Mouse: IL3, IL4, IL5, IL6, IL7, IL9, EGF, GM-CSF, TNF alphs

Rat: IL6

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.