Rat IL6R alpha ELISA Kit

Enzyme Immunoassay for the quantification of Rat IL6R alpha in Rat Serum, plasma and cell culture supernatants.

Catalog number: ARG81376

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INTRODUCTION

This gene encodes 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]

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 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 to bind to IL6R alpha captured by the first antibody. 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	1 X 96 well plate	4°C
Standard	3 X 0.5 ng/vial (Lyophilized)	4°C
Biotin-antibody conjugate concentrate	1 vials (lyophilized)	4°C
HRP-Streptavidin conjugate concentrate	1 vial (53 µl)	4°C
Diluent buffer	21 ml (Ready to use)	4°C
20X PBS	30 ml	4°C
20X Assay Buffer	20 ml	4°C
TMB substrate	10.5 ml (Ready to use)	4°C (Protect from light)
STOP solution	5.5 ml (Ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Sterile 1 x PBS
- 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.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- Briefly spin down (6000Xg for 1 min) the Standards, Biotin-antibody conjugate and HRP-streptavidin conjugate before use.
- If crystals are observed in the 20X Assay Buffer and sample diluent, warm to RT until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- A standard curve should be generated for each set of samples assayed. Thorough mixing of standards at each of dilution steps is critical to acquire a normal standard curve.
- Brief vortex samples and diluted standards for 10 sec to mix well before add to the 96 well plate.
- All reagents should be mixed by gentle inversion or swirling prior to use.

Do not induce foaming.

- Do not let strips dry, as this will inactivate active components in wells.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- HRP Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
- The Stop Solution is an acid solution, handle with caution.
- 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 for 10 min at 1500 x g at 4°C and aliquot & store samples at -20°C up to 1 month or-80°C up to 6 months. 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 & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X PBS: Dilute 20X PBS into deionized distilled water to yield 1X PBS.
- 1X Assay Buffer: Dilute 20X Assay Buffer into 1X PBS to yield 1X Assay buffer. The diluted 1X Assay Buffer can be stored at 4°C.
- 1x Biotin-antibody Conjugate: The lyophilized Biotin-antibody conjugate could be stored at 4°C to -20°C for up to 3 months. Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. Reconstitute the Biotin-antibody Conjugate with 200 µl of sterile 1 x PBS, vortex for 30 sec and keep the antibody in the vail for 5 min to completely dissolve. Centrifuge the vial for 1 min at 6000 x g before opening. Aliquot and store the antibody stock at -20°C until use. Avoid repeated freeze-thaw cycles.

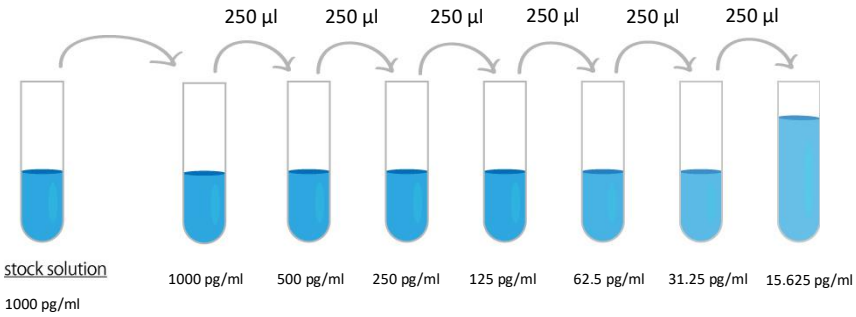
  If the entire 96-well plate is used, dilution of the 200 µl of concentrated Biotin-Conjugate solution with 10.5 ml Diluent Buffer to yield 1X Biotin-antibody Conjugate working solution.
- 1X HRP-streptavidin conjugate: Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. The stock vial includes 53 µl of HRP-streptavidin concentrate. Please confirm if the vial contains 53 µl of HRP-streptavidin concentrate before further dilution. If it is less than 53 µl, add sterile 1X PBS to reach 53 µl and vortex briefly for 10 sec. Make a 1:200 dilution of the concentrated HRP-streptavidin solution with Diluent Buffer (If the entire 96-well plate is used, add 53 µl concentrated HRP-streptavidin solution into 10.5 ml Diluent Buffer and mix thoroughly prior to the assay). The rest of undiluted HRP-streptavidin Conjugate can be stored at 4°C for up to 3 months. DO NOT FREEZE.

- Sample: Samples should be diluted with equal volume of 1 x Assay Buffer and vortex for 1 min prior to assay. If the initial assay found samples contain IL6R alpha higher than the highest standard, the samples can be diluted with 1 x Assay Buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with 1 x Assay Buffer before assay.

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

- Standards: The non-reconstituted standard can be stored at 4°C or-20°C for up to 3 months. Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. Reconstitute the standard with 0.5 ml 1 x Assay Buffer to yield a stock concentration of 1000 pg/ml. Brief vortex the vials for 30 sec and keep the standard stock in the vail for 5 min to completely dissolve. Make sure the standard is dissolved completely and then centrifuge the vial for 1 min at 6000 x g before making serial dilutions. Aliquot and store the reconstituted standard at -20°C for up 2 days.

  The 1 x Assay Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with 1X Assay Buffer 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. Brief vortex the vials for 30 sec for each standard dilution steps to mix well.



Dilute IL6R alpha standard as according to the table below:

Standard	IL6R alpha Conc. (pg/ml)	µl of 1X Assay Buffer	µl of standard
S7	1000 pg/ml	0	500 (1000 pg/ml Stock)
S6	500 pg/ml	250	250 (S7)
S5	250 pg/ml	250	250 (S6)
S4	125 pg/ml	250	250 (S5)
S3	62.5 pg/ml	250	250 (S4)
S2	31.25 pg/ml	250	250 (S3)
S1	15.625 pg/ml	250	250 (S2)
S0	0	250	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be **assayed in duplicates.**

1. Lift the plate cover from the top left and cover the wells that are not used. Brief vortex and then spin down the standards and samples for 10 sec to mix completely before applying to the plate.
2. Add **100 µl** of standards, samples and zero controls (1X Assay Buffer) **in duplicates** into wells. Incubate for **1 hour** at **room temperature**.
3. Aspirate each well and wash, repeating the process twice for a total three washes. Wash by filling each well with **1× Assay 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 Assay Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µl** of **1x Biotin-antibody Conjugate working solution** to each well. Cover the plate and incubate **1 hour** at **room temperature**.
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 **20 minutes** at **room temperature** in dark.
7. Aspirate each well and wash as step 3.
8. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate for **5-20 minutes** (depending on signal) at **room temperature** in dark.
9. Add **50 µl** of **Stop Solution** to each well. Gently tap the plate to ensure thorough mixing.
10. Read the OD with a microplate reader at **450 nm** immediately. (Optional:

it is recommended to detect background signal by reading the signal at 540-570 nm as reference wavelength).

CALCULATION OF RESULTS

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

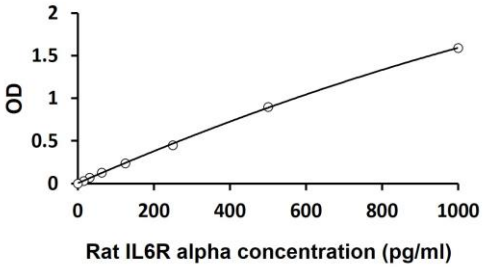
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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. 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 Rat IL6R alpha ranged from 15.6- 1000 pg/ml. The mean MDD was 3 pg/ml.

Specificity

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

Recombinant Rat ApoAI, BMP1, BMP2, BMP3, BMP4, BMP7, CRP, HGF, HSP27, IL1 alpha, IL1 RI, IL1 beta, IL1R alpha, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL13, IL15, IL17C, IL21, IL23, IFN, MMP2, sIL 2R, PDGF, PLA2G7, prolactin, TGF beta 1, TGF beta 2, TGF beta

Intra-assay and Inter-assay precision

The CV values of intra-assay was 6% and inter-assay was 9%.