Mouse IL6 (High sensitive) ELISA Kit

Enzyme Immunoassay for the quantification of Mouse IL6 in serum, plasma and cell culture supernatants.

Catalog number: ARG83819

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

This gene encodes a cytokine that functions in inflammation and the maturation of B cells. In addition, the encoded protein has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. The functioning of this gene is implicated in a wide variety of inflammation-associated disease states, including suspectibility to diabetes mellitus and systemic juvenile rheumatoid arthritis. [provided by RefSeq, Jun 2011]

Cytokine with a wide variety of biological functions. It is a potent inducer of the acute phase response. Plays an essential role in the final differentiation of B-cells into Ig-secreting cells Involved in lymphocyte and monocyte differentiation. Acts on B-cells, T-cells, hepatocytes, hematopoietic progenitor cells and cells of the CNS. Required for the generation of T(H)17 cells. Also acts as a myokine. It is discharged into the bloodstream after muscle contraction and acts to increase the breakdown of fats and to improve insulin resistance. It induces myeloma and plasmacytoma growth and induces nerve cells differentiation. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for 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 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 TMB Substrate is added to the wells and color develops in proportion to the amount of 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 IL6 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store 100X Antibody Conjugate at -20°C and other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 X 12 strips	4°C
Standard	3 X 0.2 ng/vial	4°C
Standard / Sample Diluent Buffer	20 ml (ready to use)	4°C
100X Antibody Conjugate	120 µl	-20°C
100X HRP-Streptavidin	120 µl	4°C
Antibody Diluent Buffer	30 ml (ready to use)	4°C
20X Wash Buffer	45 ml	4°C
TMB Substrate	12 ml (ready to use)	4°C (Protect from light)
STOP Solution	12 ml (ready to use)	4°C
Plate Sealer	3 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 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.
- Upon received, store 100X Antibody Conjugate at -20°C. Store other components at 2-8°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) 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 Substrate 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 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.

Note:

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

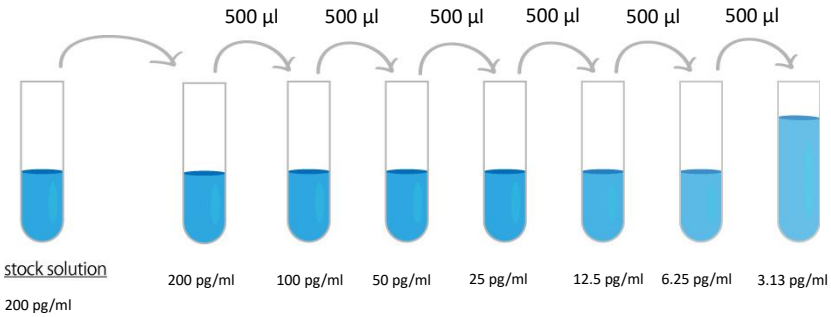
b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. The diluted Wash Buffer is stable for 4 weeks at 2°C to 8°C.
- 1X Antibody Conjugate: 20 minutes before use, dilute 100X Antibody Conjugate into Antibody Diluent Buffer to yield 1X Antibody Conjugate.
- 1X HRP-Streptavidin: 20 minutes before use, dilute 100X HRP-Streptavidin into Antibody Diluent Buffer to yield 1X HRP-Streptavidin. Keep diluted HRP-Streptavidin in dark before use.
- Sample: If initial assay results indicate that sample concentrations exceed the highest point of the standard curve, dilute the samples using the provided Standard / Sample Diluent Buffer and re-assay.

  A preliminary experiment is recommended for all sample types to determine the optimal dilution factor. For serum and plasma samples, a 1:1 dilution with the Standard / Sample Diluent Buffer (dilution factor = 2) is generally sufficient in most cases.

  Note: When calculating final concentrations, be sure to apply the appropriate dilution factor.
- Standards: Reconstitute the standard with 1 ml Standard / Sample Diluent Buffer to yield a stock concentration of 200 pg/ml. 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. 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: 200 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml, 12.5 pg/ml, 6.25 pg/ml, 3.13 pg/ml. DO NOT reuse the reconstituted standard.



Dilute IL6 standard as according to the table below:

Standard	IL6 Conc.	µl of Standard diluent	µl of standard
S7	200 pg/ml	0	1000 (200 pg/ml Stock)
S6	100 pg/ml	500	500 (S7)
S5	50 pg/ml	500	500 (S6)
S4	25 pg/ml	500	500 (S5)
S3	12.5 pg/ml	500	500 (S4)
S2	6.25 pg/ml	500	500 (S3)
S1	3.13 pg/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 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.
2. Add 100 µl of standards, samples and zero controls (Standard / Sample Diluent Buffer) into wells, gently tap the plate to mix well. Incubate for 2 h at RT.
3. Aspirate each well and wash, repeating the process four times for a total five washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer, keep the Wash Buffer in the wells for 60 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 Antibody Conjugate into each well, gently tap the plate to mix well. Cover wells and incubate for 1 hour at RT.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of HRP-Streptavidin to each well, gently tap the plate to mix well. Cover wells and incubate for 30 min at RT in dark.
7. Aspirate each well and wash as step 3.
8. Add 100 µl of TMB Reagent to each well, gently tap the plate to mix well. Incubate for 10-15 minutes at RT in dark.
9. Add 100 µl of STOP Solution to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow. Read the OD with a microplate reader at 450 nm immediately. It is recommended read

the absorbance within 15 min after adding 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.

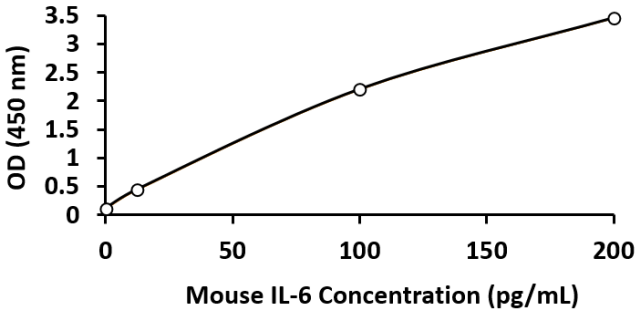
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. 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)

6. 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 Mouse IL6 ranged from 3.125 – 200 pg/mL. The mean MDD was 1.56 pg/mL.

Specificity

This assay recognizes natural and recombinant Mouse IL6.

Intra-assay and Inter-assay precision

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