Guinea Pig IL6 ELISA Kit

Guinea Pig IL6 ELISA Kit is an Enzyme Immunoassay kit for the quantification of Guinea Pig IL6 in serum, plasma and cell culture supernatants.

Catalog number: ARG82812

Package: 96 wells

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

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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. [Provide by Wikipedia: IL-6]

PRINCIPLE OF THE ASSAY

This Guinea Pig IL-6 ELISA kit is a quantitative sandwich enzyme immunoassay that measures the amount of Guinea Pig IL-6 in the samples. An antibody specific for Guinea Pig IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Guinea Pig IL-6 is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of IL-6 bound in the initial step. The color development is stopped, and the intensity of the color is measured.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all reagent at 2-8°C upon receiving. Do not use kit components past kit expiration date.

Component	Quantity	Storage information
Capture Antibody	1 vial, lyophilized	4°C
Detection Antibody	1 vial, lyophilized	4°C
Standard	3 vial, lyophilized	4°C
Streptavidin-HRP Conjugate	550 µL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- PBS, pH7.3, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.2 µm filtered.
- Assay Buffer, 0.05% Tween 20 in PBS, pH 7.3.
- Reagent Diluent, 1% Guinea Pig serum albumin in PBS, pH 7.3.
- TMB substrate
- Stop solution (1M H_2SO_4)
- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Change pipette tips between the addition of different reagent or samples.
- A thorough and consistent wash technique is essential for proper assay performance. Assay buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Assay buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at-20°C or lower. Avoid freeze-thaw cycles.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at-20°C or lower. Avoid freeze-thaw cycles.

Cell culture supernatant: Centrifuge at 300 x g for 10 minutes at 4°C to remove the cell debris.

Note:

- Samples should be diluted with four volumes of 1 x Assay Buffer and vortex for 1 minute prior to assay. If the OD value still exceeds the upper limit of the standard curve, further dilution is recommended till it falls in the detection range and the dilution factor must be used for calculation of the concentration.
- Do not use haemolytic, icteric or lipaemic specimens.
- Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **Capture Antibody:** Centrifuge at 6000 x g for 1 minute to bring down the material prior to open the vial. Refer to the lot-specific CoA for the amount supplied. Reconstitute the vial with 0.55 mL of 1X PBS. Dilute in 1X PBS without carrier protein to the working concentration indicated on the CoA. Store the vial at -20°C after reconstitution.
- **Detection Antibody:** Centrifuge at 6000 x g for 1 minute to bring down the material prior to open the vial. Refer to the lot-specific CoA for the amount supplied. Reconstitute the vial with 0.55 mL of 1X PBS. Dilute in 1X PBS to the working concentration indicated on the CoA. Store the vial at -20°C after reconstitution.
- **Streptavidin-HRP Conjugate:** Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. The vial contains 550 µL of Streptavidin-HRP conjugated to horseradish peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent. DO NOT FREEZE.
- **Standard:** Centrifuge at 6000 x g for 1 min to bring down the material prior to open the vial. Reconstitute each vial with 200 µL of Assay Buffer. Prepare 500 µL of High Standard per plate assayed at the concentration indicated on the CoA with Assay Buffer. Diluted the standard as follow.

Standard tube	IFN gamma (pg/mL)	1X Assay Buffer (µL)	Standard stock, 3000 pg/mL (µL)
S1	3000	0	500
S2	1500	250	250 of S1
S3	750	250	250 of S2
S4	375	250	250 of S3
S5	187.5	250	250 of S4
S6	93.75	250	250 of S5
S7	46.875	250	250 of S6
S0	0	500	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with **100 µL** per well of the diluted Capture Antibody and incubate for **2 hours** at **room temperature**.
2. Aspirate each well and wash with **300 µL** of **Assay Buffer** per well and remove any remaining Assay Buffer by aspiration or by inverting the plate and blotting it against clean paper towel.
3. Blocks plates by adding **150 µL** of **Reagent Diluent** to each well. Incubate for **1 hour** at **room temperature**.
4. Wash the plate as shown in Step 2.

ELISA procedure

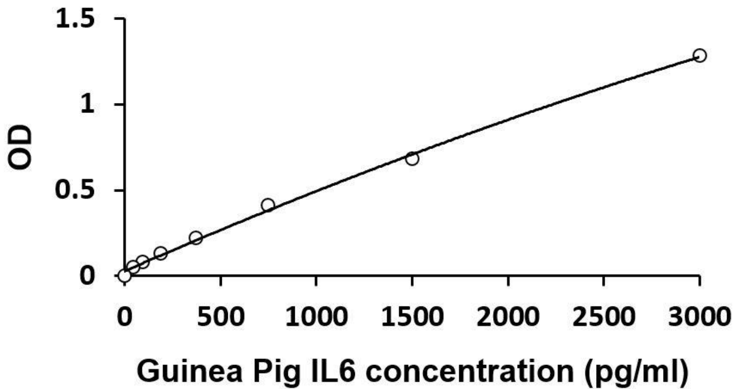
1. Add **100 µL** of **diluted samples** or **each diluted Standard** into respective wells.
2. Cover the plate and incubate for **60 mins** at **room temperature**.
3. Aspirate each well and wash, repeating the process 2 time for a total 3 washes. Wash by filling each well with **Assay Buffer (300 µL)** 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 Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **Working Dilution of Detection Antibody** to each well. Mix well by repeated pipetting.
5. Cover the plate and incubate for **60 mins** at **room temperature**.
6. Aspirate and wash plate as in step 3.
7. Add **100 µL** of **Working Dilution of Streptavidin-HRP Conjugate** to each well.
8. Cover the plate and incubate for **20 mins** at **room temperature** in the dark.
9. Aspirate and wash plate as in step 3.
10. Add **100 µL** of **TMB Substrate** in each well.
11. Incubate for **5-20 mins** at **room temperature** in the dark.
12. Add **50 µL** of **Stop Solution** to each well to stop the reaction.
13. Read the absorbance with a plate reader at **O.D. 450 nm.**

CALCULATION OF RESULTS

1. Subtract zero point (S0) from all standards and unknowns to determine corrected absorbance.
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. 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.
4. 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)
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 figures demonstrate typical results with the Guinea Pig IL-6 ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

23.5 pg/mL

Precision

Intra Assay CV: 7.0%; Inter Assay CV: 8.0%