arigoQIK™ Rat IL4 ELISA Development Kit

ARG83549 arigoQIK™ Rat IL4 ELISA Development Kit is designed for the development of sandwich ELISA to measure Interleukin 4 in serum, plasma, cell culture supernatants.

Catalog number: ARG83549

Package: 1 kit (5 plates)
(15 plates)

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INTRODUCTION

The protein encoded by this gene is a pleiotropic cytokine produced by activated T cells. This cytokine is a ligand for interleukin 4 receptor. The interleukin 4 receptor also binds to IL13, which may contribute to many overlapping functions of this cytokine and IL13. STAT6, a signal transducer and activator of transcription, has been shown to play a central role in mediating the immune regulatory signal of this cytokine. This gene, IL3, IL5, IL13, and CSF2 form a cytokine gene cluster on chromosome 5q, with this gene particularly close to IL13. This gene, IL13 and IL5 are found to be regulated coordinately by several long-range regulatory elements in an over 120 kilobase range on the chromosome. IL4 is considered an important cytokine for tissue repair, counterbalancing the effects of proinflammatory type 1 cytokines, however, it also promotes allergic airway inflammation. Moreover, IL-4, a type 2 cytokine, mediates and regulates a variety of human host responses such as allergic, anti-parasitic, wound healing, and acute inflammation. This cytokine has been reported to promote resolution of neutrophil-mediated acute lung injury. In an allergic response, IL-4 has an essential role in the production of allergen-specific immunoglobin (Ig) E. This pro-inflammatory cytokine has been observed to be increased in COVID-19 (Coronavirus disease 2019) patients, but is not necessarily associated with severe COVID-19 pathology. Two alternatively spliced transcript variants of this gene encoding distinct isoforms have been reported. [provided by RefSeq, Aug 2020]

PRINCIPLE OF THE ASSAY

This pair employs the quantitative sandwich enzyme immunoassay technique. Coating specific IL4 antibody on a microtiter plate. Standards or samples are pipetted into the wells and any IL4 present is bound by the immobilized antibody. After washing away any unbound substances, a IL4 Detection is added to each well and incubate. Following a washing to remove unbound substances, HRP-Streptavidin Solution is added to each microplate well and incubated. After washing away any unbound antibody, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of GM-CSF 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. The concentration of IL4 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 100X Human GM-CSF Detection at ≤ -20°C.

Store other components at 2-8°C at all times. Use the kit before expiration date.

Component	Package (5 plates)	Package (15 plates)	Storage information
100X Interleukin 4 Capture Antibody	1 vial	3 vial	4°C
Interleukin 4 Standard	1 vial	3 vial	4°C
100X Interleukin 4 Detection Antibody	1 vial	3 vial	≤ -20°C
100X HRP-Streptavidin Solution	1 vial	3 vial	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Phosphate buffered saline (PBS)
- Wash buffer
- Antibody Diluent Buffer, Standard / Sample Diluent Buffer
- Blocking Buffer
- Substrate Solution, Stop Solution
- Deionized or distilled water
- 96-well plate
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microplate reader capable of reading at 450 nm
- Microtiter plate washer (optional)
- For commercial reagents required for arigoQIK™ ELISA Development Kit, please refer ARG83524 Integral Reagent Kit (ELISA Development Kit)

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

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.
c) Avoid disturbing the white buffy layer when collection serum / plasma sample.

REAGENT PREPARATION

- **Phosphate buffered saline (PBS)**: Prepared with 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.5 mM KH_2PO_4. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.
- **10X Wash Buffer**: Add Tween-20 to the previously prepared PBS to achieve a final concentration of 0.5%. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.

  Dilute 10X Wash Buffer with distilled water to yield 1X Wash Buffer. The diluted 1X Wash Buffer is stable for 4 weeks at 2°C to 8°C.
- **Standard / Sample Diluent Buffer**: Add BSA and Tween-20 to the previously prepared PBS to achieve a final concentration of 1% BSA and 0.05% Tween-20. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.
- **Antibody Diluent Buffer**: Add BSA and Tween-20 to the previously prepared PBS to achieve a final concentration of 1% BSA and 0.1% Tween-20. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.
- **Blocking Buffer**: Add BSA and Tween-20 to the previously prepared PBS to achieve a final concentration of 1% BSA and 0.05% Tween-20. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.

- **1X Interleukin 4 Capture**: It is recommended to prepare this reagent immediately prior to use and use it within 15 min after preparation. Dilute 100X Interleukin 4 Capture with prepared **PBS** previously, to yield 1X Interleukin 4 Capture. The diluted 1X Interleukin 4 Capture is stable for 8 weeks at 2-8°C. For long-term storage, aliquot and store at-20°C to-70 °C.
- **1X Interleukin 4 Detection**: It is recommended to prepare this reagent immediately prior to use and use it within 15 min after preparation. Dilute 100X Interleukin 4 Detection with prepare **Antibody Diluent Buffer** previously, to yield 1X Interleukin 4 Detection. The diluted 1X Interleukin 4 Detection is stable for 8 weeks at 2-8°C. For long-term storage, aliquot and store at-20°C to-70 °C.
- **1X HRP-Streptavidin Solution**: It is recommended to prepare this reagent immediately prior to use and use it within 15 min after preparation. Dilute 100X HRP-Streptavidin Solution with prepare **Antibody Diluent Buffer** previously, to yield 1X HRP-Streptavidin Solution. The diluted 1X HRP-Streptavidin Solution is stable for 4 weeks at 2-8°C. Do NOT freeze 1X HRP-Streptavidin Solution.
- **Sample**: If the initial assay found samples contain proteins higher than the highest standard, the samples can be diluted with Standard / Sample Diluent Buffer and then re-assay the samples.

Standard: Centrifuge the un-reconstituted standard at 6000 x g for 1 minute to bring down the material prior to open the vial. Reconstituted Standard as label on the Standard vial. The reconstituted Standard stock concentration would be 10000 pg/ml. Vortex for few seconds (Do not induce foaming) and allow it to sit for 15 minutes as stock. The Standard stock is stable for 4 weeks at 2-8°C. For long-term storage, aliquot and store at -20°C to -70 °C.

- 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 with **Standard / Sample Diluent Buffer**. Diluted the standard as below:

Standard tube	IL4 (pg/mL)	Standard / Sample Diluent Buffer (µL)	Standard stock (µL)
Pre1	1000	900	100 (10000 pg/ml Standard Stock)
S1	500	500	500 (1000 pg/ml Pre1)
S2	250	500	500 of S1
S3	125	500	500 of S2
S4	63	500	500 of S3
S5	31	500	500 of S4
S6	15.63	500	500 of S5
S7	7.81	500	500 of S6
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature before use.

1. Add **100 µL** of **1X Interleukin 4 Capture** into respective well.
2. Cover the plate and incubate for **16-20 hours** at 4°C.
3. Remove the cover and discard the liquid in the wells.
4. Add **250 µl** of **Blocking Buffer** to each well.
5. Cover the plate and incubate for **2 hours** at room temperature.
6. Remove the cover and discard the liquid in the wells.
7. Add **100 µL** of **samples** and **each diluted Standard** into respective well.
8. Add **50 µL** of **1X Interleukin 4 Detection** into each well.
9. Cover the plate and incubate for **1 hour** at room temperature on a microplate shaker set at 500 rpm.
10. Aspirate each well and wash, repeating the process 4 time for a **total 5 washes.** Wash by filling each well with **1X Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or auto-washer. 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.
11. Add **100 µL** of **1X HRP-Streptavidin Solution** into each well.
12. Cover the plate and incubate for **30 min** at room temperature in the dark on a microplate shaker set at 500 rpm.
13. Aspirate each well and **wash** plate **as step 10**.
14. Add **100 µL** of **TMB Substrate** in each well.
15. Incubate for **5-30 mins** at room temperature in the dark.
16. Add **100 µL** of **Stop Solution** to each well to stop the reaction.

17. Read the absorbance with a plate reader at O.D. 450 nm within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control 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)
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.