arigoQIK® Human RANTES ELISA Development Kit

ARG83764 arigoQIK® Human RANTES ELISA Development Kit is designed for the development of sandwich ELISA to measure Human RANTES in serum, plasma, cell culture supernatants.

Catalog number: ARG83764

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

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

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MANUFACTURED BY:

arigo Bolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

This gene is one of several chemokine genes clustered on the q-arm of chromosome 17. Chemokines form a superfamily of secreted proteins involved in immunoregulatory and inflammatory processes. The superfamily is divided into four subfamilies based on the arrangement of the N-terminal cysteine residues of the mature peptide. This chemokine, a member of the CC subfamily, functions as a chemoattractant for blood monocytes, memory T helper cells and eosinophils. It causes the release of histamine from basophils and activates eosinophils. This cytokine is one of the major HIV-suppressive factors produced by CD8+ cells. It functions as one of the natural ligands for the chemokine receptor chemokine (C-C motif) receptor 5 (CCR5), and it suppresses in vitro replication of the R5 strains of HIV-1, which use CCR5 as a coreceptor. Alternative splicing results in multiple transcript variants that encode different isoforms. [provided by RefSeq, Jul 2013]

PRINCIPLE OF THE ASSAY

This pair employs the quantitative sandwich enzyme immunoassay technique. Coating specific Human RANTES Capture Antibody on a microtiter plate. Standards or samples are pipetted into the wells and any Human RANTES present is bound by the immobilized antibody. After washing away any unbound substances, a Human RANTES Detection Antibody is added to each well and incubate. Following a washing to remove unbound substances, HRP-Streptavidin 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 Human RANTES 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 Human RANTES in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

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

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

MATERIALS REQUIRED BUT NOT PROVIDED

- Phosphate buffered saline (PBS)
- Wash buffer
- Antibody Diluent Buffer, Standard / Sample Diluent Buffer
- Blocking Buffer
- TMB Substrate, 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.
- **20X 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 20X 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 Human RANTES Capture Antibody: It is recommended to prepare this reagent immediately prior to use and use it within 15 min after preparation. Dilute 100X Human RANTES Capture Antibody with prepared PBS previously, to yield 1X Human RANTES Capture Antibody. The diluted 1X Human RANTES Capture Antibody is stable for 8 weeks at 2-8°C. For long-term storage, aliquot and store at-20°C to-70 °C.
- 1X Human RANTES Detection Antibody: It is recommended to prepare this reagent immediately prior to use and use it within 15 min after preparation. Dilute 100X Human RANTES Detection Antibody with prepare Antibody Diluent Buffer previously, to yield 1X Human RANTES Detection Antibody. The diluted 1X Human RANTES Detection Antibody 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: It is recommended to prepare this reagent immediately prior to use and use it within 15 min after preparation. Dilute 100X HRP-Streptavidin with prepare Antibody Diluent Buffer previously, to yield 1X HRP-Streptavidin. The diluted 1X HRP-Streptavidin is stable for 4 weeks at 2-8°C. Do NOT freeze 1X HRP-Streptavidin.
- 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 at room temperature 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	Human RANTES (pg/mL)	Standard / Sample Diluent Buffer (µL)	Standard stock (µL)
Pre1	1000	396	44 (10000 pg/mL Standard Stock)
S1	500	220	220 (1000 pg/mL Pre1)
S2	250	220	220 of S1
S3	125	220	220 of S2
S4	62.5	220	220 of S3
S5	31.25	220	220 of S4
S6	15.63	220	220 of S5
S7	7.81	220	220 of S6
S0	0	220	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature before use.

1. Add 100 µL of 1X Human RANTES Capture Antibody 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 Human RANTES Detection Antibody 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. 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.
11. Add 100 µL of 1X HRP-Streptavidin 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 5-15 min 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 15 min 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.