Monkey RANTES ELISA Kit

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

Catalog number: ARG82820

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

CCL5 (RANTES) 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]

Chemoattractant for blood monocytes, memory T-helper cells and eosinophils. Causes the release of histamine from basophils and activates eosinophils. May activate several chemokine receptors including CCR1, CCR3, CCR4 and CCR5. One of the major HIV-suppressive factors produced by CD8+ T-cells. Recombinant RANTES protein induces a dose-dependent inhibition of different strains of HIV-1, HIV-2, and simian immunodeficiency virus (SIV). The processed form RANTES(3-68) acts as a natural chemotaxis inhibitor and is a more potent inhibitor of HIV-1-infection. The second processed form RANTES(4-68) exhibits reduced chemotactic and HIV-suppressive activity compared with RANTES(1-68) and RANTES(3-68) and is generated by an unidentified enzyme associated with monocytes and neutrophils (PubMed:16791620, PubMed:1380064,

PubMed:8525373, PubMed:9516414, PubMed:15923218). May also be an agonist of the G protein-coupled receptor GPR75, stimulating inositol trisphosphate production and calcium mobilization through its activation. Together with GPR75, may play a role in neuron survival through activation of a downstream signaling pathway involving the PI3, Akt and MAP kinases. By activating GPR75 may also play a role in insulin secretion by islet cells (PubMed:23979485). [UniProt]

PRINCIPLE OF THE ASSAY

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

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	8 x 12 strips	4°C
Standard	3 X 0.5 ng/ml (Lyophilized)	4°C
Biotin-conjugated antibody concentrate	1 vials (lyophilized)	4°C
HRP-Streptavidin concentrate	55 µL	4°C
20X Assay Buffer	20 mL	4°C
20X PBS	25 mL	4°C
TMB Substrate	10.5 mL	4°C
Stop Solution	5.5 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 540-570 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Sterile 1 x PBS
- Automated microplate washer (optional)

TECHNICAL NOTES 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-conjugated antibody and HRP-Streptavidin before use.
- If crystals are observed in the 20X Assay Buffer or 20X PBS 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-Streptavidin concentrate 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.

Note:

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

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

REAGENT PREPARATION

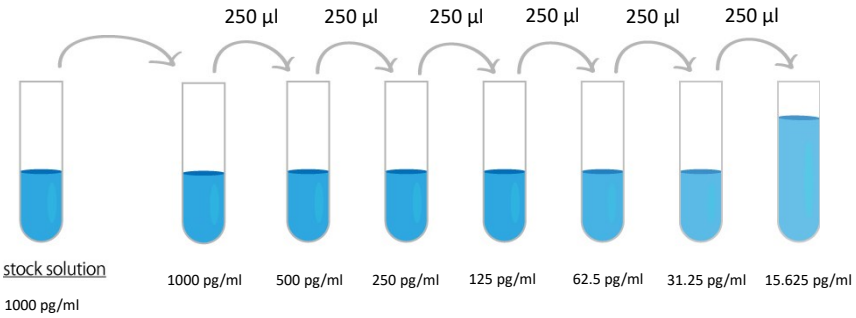
- **1X PBS:** Dilute **20X PBS** into **distilled water** to yield 1 X PBS (E.g., add 25 mL of 20X PBS into 475 mL of distilled water to a final volume of 500 mL).
- **1X Assay Buffer:** Dilute **20X Assay Buffer** into **1X PBS** to yield 1X Assay Buffer (E.g., add 20 mL of 20X Assay Buffer into 380 mL of 1X PBS to a final volume of 400 mL).
- **1x Biotin-conjugated antibody:** The lyophilized Biotin-conjugated antibody concentrate could be stored at 4°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 concentrated Biotin-conjugated antibody stock with **200 µl** of **sterile 1 x PBS**, vortex briefly for 30 sec and keep the antibody in the vail for few minutes 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 stock** solution with **10.5 ml** of **1X PBS** to yield 1X Biotin-conjugated antibody working solution.
- **HRP-Streptavidin:** Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. The stock vial includes **55 µl** of HRP-streptavidin concentrate. Please confirm if the vial contains 55 µl of HRP-streptavidin concentrate before further dilution. If it is less than 55 µl, add sterile 1X PBS to reach 55 µl and vortex briefly for 10 sec. Make a **1:200 dilution** of the concentrated HRP-streptavidin solution with **1X PBS** (If the entire 96-well plate is used, add **53 µl** of **concentrated HRP-streptavidin**

solution into 10.5 ml of 1X PBS and mix thoroughly prior to the assay). The rest of undiluted HRP-Streptavidin can be stored at 4°C for up to 3 months. DO NOT FREEZE.

- Sample: Samples should be diluted with equal volumes of 1 x Assay Buffer and vortex for 1 min prior to assay. If the initial assay found samples contain RANTES 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 to 2 days.

  The 1 x Assay Buffer serves as zero standard (0 ng/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 and 15.625 pg/ml,. Brief vortex the vials for 30 sec for each standard dilution steps to mix well.



Dilute RANTES standard as according to the table below:

Standard tube	RANTES (pg/mL)	1X Assay Buffer (µL)	Standard stock, 1000 pg/mL (µL)
S1	1000	0	500
S2	500	250	250 of S1
S3	250	250	250 of S2
S4	125	250	250 of S3
S5	62.5	250	250 of S4
S6	31.25	250	250 of S5
S7	15.625	250	250 of S6
S0	0	500	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 **1.5 hour** at **room temperature**.
3. Aspirate each well and wash, repeating the process once for a **total two 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-conjugated antibody working solution** to each well. Cover the plate and incubate **1.5 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** but wash for a **total four washes.**
8. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate for **5-30 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.

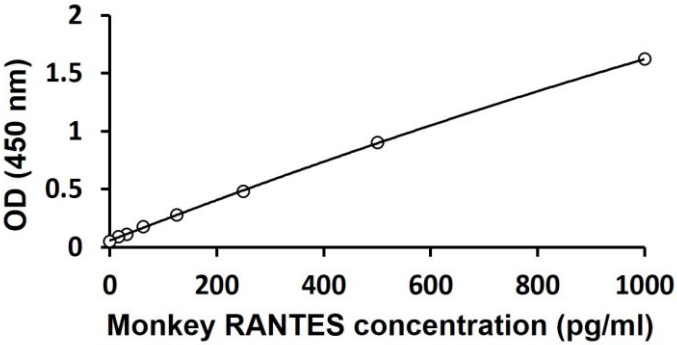
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. 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 Monkey RANTES ranged from 15.625 - 1000 pg/ml. The mean MDD was 7.8 pg/ml.

Specificity

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

Recombinant Monkey ApoAI, BMP7, CCL2, CRP, FGF acidic, HGF, HSP27, IL1 alpha, IFN gamma, IGF1, MMP2, PDGF, PLA2G7, serpin E1, TGF beta 1, TLR3, TNF alpha and VEGF.

Intra-assay and Inter-assay precision

The CV values of intra-assay was 6% and inter-assay was 10%.