



## Human Rantes ELISA

Catalog Number EA-0506

(For Research Use Only)

### Introduction

RANTES is a chemotactic protein for T cells, eosinophils, and basophils. RANTES is produced by circulating T-cells. The synthesis of RANTES is induced by TNF-alpha and IL1-alpha. It plays an active role in recruiting leukocytes into inflammatory sites. With the help of particular cytokines that are released by T cells, Rantes also induces the proliferation and activation of certain natural-killer (NK) cells to form CC-Chemokine-activated killer cells. It is also an HIV-suppressive factor released from CD8+ T cells. RANTES increases the adherence of monocytes to endothelial cells.

### Principle of the assay

Rantes ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-human Rantes for immobilization on the microtiter wells and biotinylated rabbit anti-human Rantes antibodies along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the Rantes molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of Rantes is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Diagram of ELISA

### Materials provided with the kit

- 8x12 96-well microplate coated with rabbit anti-human Rantes antibodies (4°C).
- Biotin labeled rabbit anti-human Rantes antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant human Rantes standard (1000ng/ml) (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (RT)
- Substrate (4°C).
- Stop Solution (4°C).

### Material required but not provided

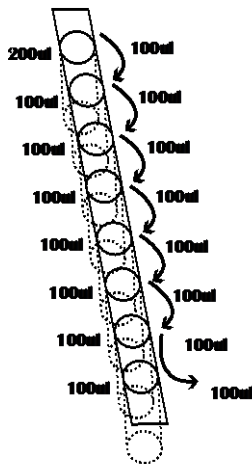
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer  
40ml 5x Assay wash buffer  
160ml ddH<sub>2</sub>O
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 100 times of Human recombinant Rantes (400ng/ml) with 1X Diluent buffer to 4000pg/ and then 2-fold serial dilutions by adding 2ul Human Recombinant Rantes in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- Dilute 400 times of biotin labeled rabbit anti-human Rantes antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

## Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of wells are well sealed.
2. Add 100µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.



- a. Add 200ul 1X Diluent buffer to the 1<sup>st</sup> well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
- b. Add appropriate amount of protein recombinant (follow instruction in "Reagent Preparation")
- c. Mix dilutions in 1<sup>st</sup> well and transfer 100ul from the 1<sup>st</sup> well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100 µl of diluted biotin-labeled rabbit anti-human Rantes antibodies to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.

7. Repeat the aspiration/wash as in step 3.
8. Add 100µl of substrate to each well and incubate for 5-30 minutes.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.