PROMPT
Proteins for Research

ISO 9001:2015 ISO 13485:2016

Human RANTES (Regulated On Activation In Normal T-Cell Expression and Secreted) ELISA Kit

Cat: OPK1179

For research use only. Not intended for diagnostic use.

Detection Range: 1.56 – 100ng/mL

Sensitivity: 0.15ng/mL

Repeatability: Coefficient of variation is 10%

Kit Components &Storage

An unopened kit can be stored at 2-8℃ for 1 year. If the opened kit is not used up, store the items separately according to the following conditions.

Reagents	Quantity		Storage Condition
	96T	48T	
Micro ELISA Plate (Dismountable)	12 strips x 8 wells	6 strips x 8 wells	4℃ (6 months) -20℃ (12 months)
Reference Standard	2vials	1vial	4℃ (6 months) -20℃ (12 months)
Concentrated Biotinylated Detection Ab (100×)	120µL	60µL	4℃ (6 months) -20℃ (12 months)
Concentrated HRP Conjugate (100×)	120µL	60µL	4℃ (6 months) -20℃ (12 months)
Reference Standard & Sample Diluent	20mL	20mL	4℃ (12 months)
Biotinylated Detection Ab Diluent	13mL	13mL	4℃ (12 months)
HRP Conjugate Diluent	13mL	13mL	4℃ (12 months)
Concentrated Wash Buffer (25×)	30mL	30mL	4℃ (12 months)
Substrate Reagent	10mL	10mL	4℃ (12 months, store in Dark)
Stop Solution	10mL	10mL	4℃ (12 months)
Plate Sealer	5pieces	5pieces	RT

Origin

Materials Required, Not Supplied

1. Microplate reader capable of measuring absorbance at 450 ± 10nm.
2. High-speed centrifuge.
3. Electro-heating standing-temperature cultivator.
4. Absorbent paper.
5. Double distilled water or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2µL to 1mL volumes.

Safety Notes

1. This kit is only used for lab research and development and should not be used for human or animals.
2. Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with Reagents. In case of contact, wash thoroughly with water.

Test Principle

This kit uses the Sandwich-ELISA principle. The microtiter plate strips has been pre- coated with an affinity purified antibody to Human RANTES. Standard or Samples containing Human RANTES are added to the plate wells and reacted with capture antibody. A second anti-Human RANTES antibody labeled biotin is then added and binds to Human RANTES captured on the plate. After that, Streptavidin-Horseradish Peroxidase (SA-HRP) is added to form a sandwich complex of solid phase antibody-Human RANTES-biotin labeled antibody-SA-HRP. And then, TMB substrate solution is added to all wells and incubated. An enzyme-catalyzed reaction generates a blue colour in the solution, thereafter, stop solution is added to stop the substrate reaction and the colour turns yellow. The yellow solution is read at a wavelength of 450nm. The concentration of Human RANTES in the samples is then calculated from the OD value by establishing a standard curve.

Sample Collection and Storage

Serum - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000 × g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000 × g and 2- 8°C for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900µL lysis buffer is added in 100mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 × g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

Cell lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000 × g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 × g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 × g at 2-8°C for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Feces - Dry feces were collected as much as possible, weighing more than 50mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900µL lysis buffer is added in 100mg feces), sonicated (or mashed) and centrifuged at 5000 × g for 10 minutes, where the supernatant was collected for testing.

Cell culture supernatants and other biological fluids - Centrifuge samples at 1000 × g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

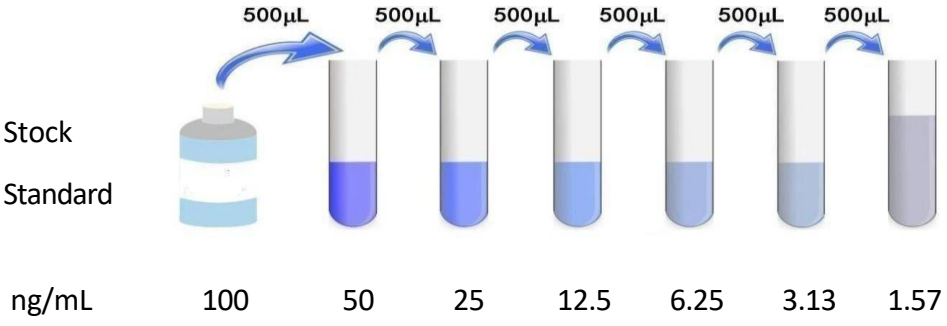
Cerebrospinal fluid (CSF) - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Notes

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination. Avoid repeated freeze- thaw cycles.
2. Sample hemolysis will influence the result, so it should not be used.
3. When performing the assay, bring samples to room temperature.

Reagent Preparation

1. Allow all reagents to return to room temperature (18-25℃) before use.
2. **Wash Buffer:** Dilute 20mL of Concentrated Wash Buffer with 480mL of deionized or distilled water to prepare 500mL of Wash Buffer.
3. **Standard working solution:** Centrifuge the Reference Standard at 10000 × g for 1 minute. Add 1mL of Reference Standard & Sample Diluent, let it stand for 10 minutes and then mix gently, that is 100ng/mL of standard working solution. Prepare 7 EP tubes, add 500µL standard and sample diluent to each tube, take 500µL from 100ng/mL to make 50ng/mL, and so on, the last tube is blank (no more liquid needs to be drawn from the penultimate tube). Use it now.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent.
5. **HRP Conjugate working solution:** Calculate the required amount before the experiment (100µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent.

Experimental Operation Tips

1. Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
3. After adding the Substrate Reagent. The reaction time can be shortened or extended according to the actual colour change, but not more than 30 minutes.
4. Adding the stop solution should be done in the same order as the substrate solution.

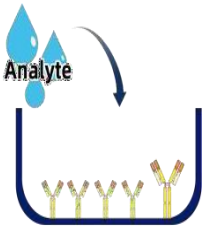
Notes

1. The detection range of the kit is different from the concentration range of the substance to be measured in the sample. It is recommended to estimate the concentration by looking up the reference before the experiment, and determine the actual concentration by pre-experiment. If the concentration is too high or too low, dilute or concentrate the sample appropriately.
2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
3. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.
4. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility to cause a deviation due to the introduced chemical substance.
5. Samples should be used within 6 days when stored at 2-8℃, otherwise samples must be stored at -20℃ (≤ 1 month) or -80℃ (≤ 3 months). Avoid repeated freeze-thaw cycles.
6. With the standard product concentration as the horizontal coordinate and OD value as the vertical coordinate, the standard curve is formed by using the nonlinear four parameter logistic (4-PL) and the sample concentration is calculated by the curve.

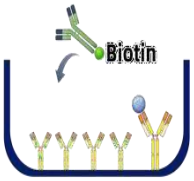
Assay Procedure

1. The Micro ELISA Plate slats to be used were removed from the plate frame and the remaining slats were returned to the aluminum foil bag containing the desiccants and then resealed for storage.
2. Determine wells for diluted standard, blank and sample. Add 100µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Cover the plate with the sealer provided in the kit. Incubate for 90 minutes at 37℃.
   Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
3. Aspirate and wash plate for 3 times. Immediately add 100µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.
4. Decant the solution from each well add 300µL of wash buffer to each well. Soak for 30 seconds and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.
   Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
5. Add 100µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 minutes at 37℃.
6. Decant the solution from each well, repeat the wash process for 3 times as conducted in step 4.
7. Add 100µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light.
   Note: the reaction time can be shortened or extended according to the actual colour change, but not more than 30 minutes. Preheat the Microplate Reader for about 15 minutes before OD measurement.
8. Add 50µL of Stop Solution to each well.
   Note: adding the stop solution should be done in the same order as the substrate solution.
9. Determine the optical density (OD value) of each well at once with a micro plate reader set to 450nm.

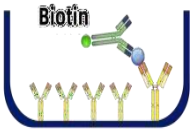
Summary for Procedure



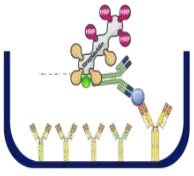
1. Add 100µL standard or sample to the wells. Incubate for 90 minutes at 37°C, Aspirate and wash plate for 3 times.



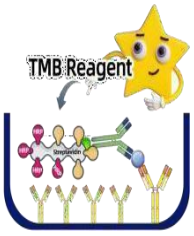
2. Immediately add 100µL Biotinylated Detecting Ab working solution to each well. Incubate for 60 minutes at 37°C.



3. Aspirate and wash the plate for 3 times.

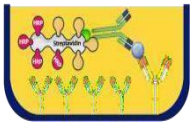


4. Add 100µL HRP conjugate working solution. Incubate for 30 minutes at 37°C.



5. Aspirate and wash the plate for 3 times. Add 100µL Substrate Reagent. Incubate for 15 minutes at 37°C.

6. Add 50µL Stop Solution.

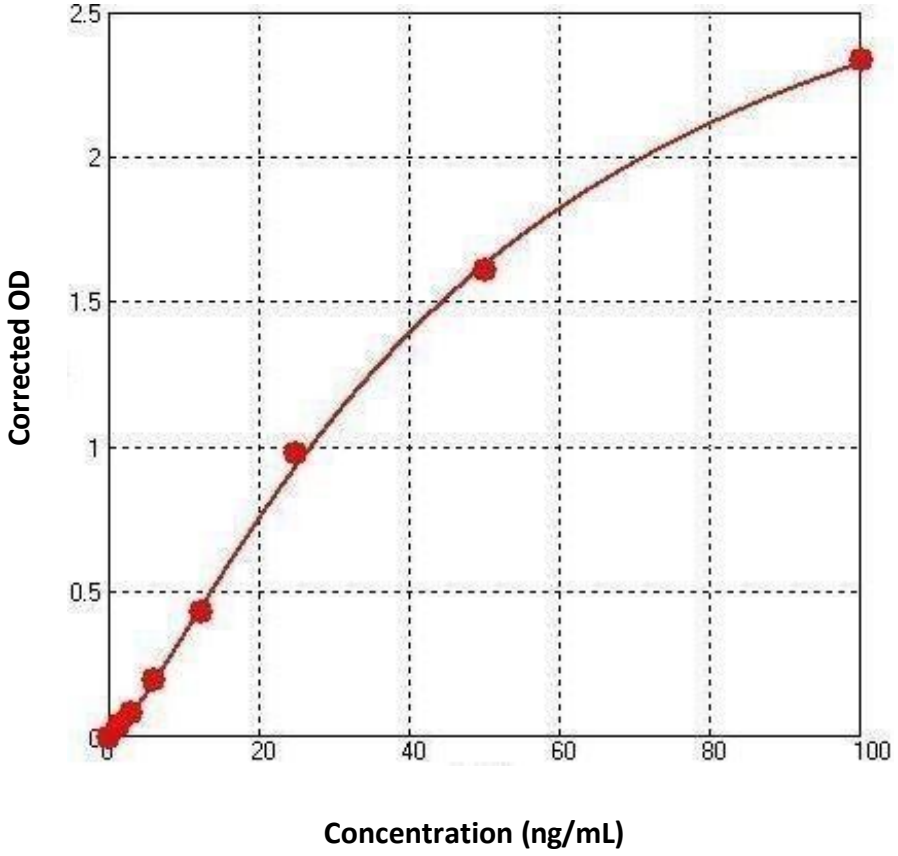


7. Read at 450nm immediately and calculate results.

Calculation of Results

For each test, a standard curve must be set for each plate. The standard curves below are for example purposes only.

Concentration (ng/mL)	OD_{450nm}	Corrected OD
100	2.395	2.333
50	1.671	1.609
25	1.042	0.98
12.5	0.492	0.43
6.25	0.26	0.198
3.13	0.144	0.082
1.57	0.102	0.04
0	0.062	0



Note: this graph is for reference only

Sensitivity

The minimum detection concentration of the Human RANTES detected by this kit is usually less than the 0.15ng/mL. The sensitivity was determined by adding two standard deviations to the mean OD value of twenty zero standard replicates and calculating the corresponding concentrations.

Precision

Mean coefficient of variation for Intra-Assay and Inter-Assay: 3 samples with low, middle and high level concentration were tested for repeat multiple times, respectively. The results showed that the coefficient of variation of the kits was less than 10%, which met the precision quality control standard.

Specificity

This method has high sensitivity and specificity for the detection of Human RANTES. No significant cross-reactivity was observed between the Human RANTES and its analogues.

Recovery

The known concentration of Human RANTES was added to different samples respectively, and the recovery experiment was conducted. The results showed that the recovery range and average recovery rate of the kit were 80-120%, which met the recovery quality control standard.

Linearity

Dilute linear experiments were performed on samples with Human RANTES to evaluate the linearity of the kit. The results showed that the kit linear range (%) was 80-120%, which met the linear quality control standard.

Limitations of the Procedure

1. This kit is for laboratory scientific research only, we will not be responsible for any consequences if this kit is used for clinical diagnosis or any other procedures.
2. Due to the uncertainty of its validity, this kit may not be suitable for testing some special experimental samples, such as gene knockout experiments.
3. This kit should be used before its expiration date, and please strictly follow the instructions for storage.
4. Different manufacturer's kits or testing the same analyte by other methods may produce inconsistent results because we do not compare our products with those of other manufacturers.
5. Since the antibodies used in the kit are usually prepared from recombinant proteins as immunogen, and recombinant proteins can be limited by different fragmentation, expression and purification systems, it is not recommended to use this kit to detect recombinant proteins.
6. In order to get the best experimental results, please use only the reagents provided by the manufacturer, and do not mix reagents from different batches.
7. Due to the existing conditions and the limitations of science and technology, we cannot fully identify and analyze the raw materials provided by the supplier comprehensively. Therefore, the kit may have some quality and technical risks.
8. The possibility of interference cannot be excluded before all factors are tested in the ELISA immunoassay.
9. In order to obtain reproducible results, each step in the experiment should be controlled and variations in sample collection, handling and storage may also lead to differences in sample measurements.
10. Although each kit passes rigorous quality testing, differences in measured values between batches of kits can still be caused by factors such as shipping conditions and different laboratory equipment.

Troubleshooting & Solutions

Problem	Possible Causes	Subsequent Actions
Poor standard curve	Improper standard dilution	Ensure that standards are dissolved and diluted in the recommended manner
	Inaccurate pipetting	Periodically calibrate pipettes and check the pipette tips
	Evaporate the reaction solution	Seal the enzyme plate with plate sealer
	Incomplete plate washing	Adequate washing times and the amount of washing solution added
	Foreign matter in the bottom of wells	Clean the bottom of the plate before reading
Weak or no colour development	Insufficient reaction of reagents	Ensure incubation time and incubate at the recommended temperature
	Inadequate reagent volumes	Check the pipette and follow the steps strictly to operate
	Improper dilution	Check the reagent dilution process
	Inactivation of enzyme conjugate	Mix conjugate and substrate, check by colour development
	No stop solution added	Add appropriate amount of stop solution
	Waiting too long time to read	Read the plate in time
High background	Contaminated chromogenic solution	Replace chromogenic solution
	Colouring time is too long	Control the colouring time
	Wrong dilution of reaction reagent	Use the recommended dilution
	Inadequate washing of the plate	Adequate washing times and the amount of washing solution added