

Human RANTES Instant ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of human RANTES

Catalog Number BMS287-2INST (128 tests)

Pub. No. MAN0016655 Rev. A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Human RANTES Instant ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human RANTES in cell culture supernatants, human serum, plasma, amniotic fluid, or other body fluids.

Summary

Chemokines are a group of cytokines that are responsible for the influx of blood cells, including T- an B-lymphocytes, monocytes, neutrophils, eosinophils and basophils, in allergic and other inflammatory conditions.

The activity of the chemokine RANTES (Regulated upon Activation, Normal T-cell Expressed and presumably Secreted) however is not restricted merely to chemotaxis. It is a powerful activator, a feature potentially relevant in a range of inflammatory disorder, both in acute and chronic inflammatory reactions. RANTES together with MCP-1 (monocyte chemoattractant protein-1) and related molecules constitute the C-C class of the β chemokine supergene family. These low molecular weight molecules act on leukocytes through selective receptors and are known to function also in leukocyte maturation, trafficking, and homing of these cells.

For literature update refer to our website.

Principles of the test

An anti-human RANTES polyclonal coating antibody is adsorbed onto microwells. Human RANTES present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated polyclonal anti-human RANTES antibody binds to human RANTES captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human RANTES.

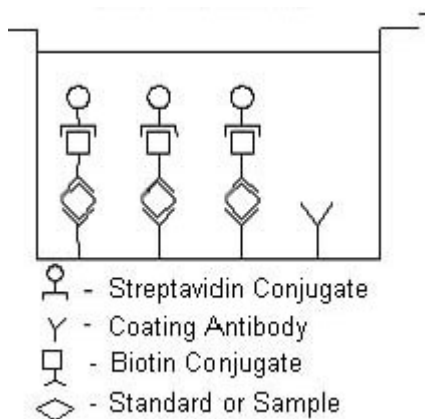


Fig. 1 First incubation

Following incubation unbound biotin conjugated anti human RANTES and Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

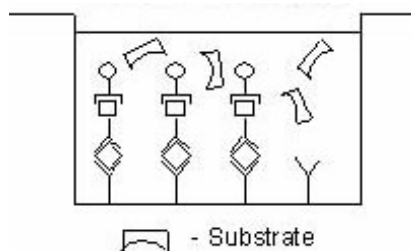


Fig. 2 Second incubation

A colored product is formed in proportion to the amount of soluble human RANTES present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human RANTES standard dilutions and human RANTES sample concentration determined.

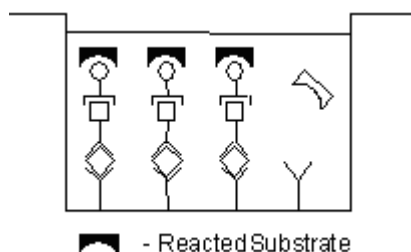


Fig. 3 Stop reaction

Reagents provided

- 1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with Polyclonal Antibody to human RANTES, Biotin-Conjugate (anti-RANTES polyclonal antibody), Sample diluent and Streptavidin-HRP, lyophilized
- 2 aluminum pouches with a human RANTES Standard curve (colored)
- 1 bottle (25 mL) Wash Buffer Concentrate 20x (phosphate-buffered saline with 1% Tween™ 20)
- 1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)
- 1 vial (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20 and 10% BSA)
- 1 vial (15 mL) Stop Solution (1M Phosphoric acid)
- 2 adhesive Plate Covers

Storage instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2°C and 8°C. Expiry of the kit and reagents is stated on labels.

The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

Sample collection

Cell culture supernatants, human serum, and plasma were tested with this assay. Other biological samples might be suitable for use in the assay. Remove the serum or plasma from the clot or red cells as soon as possible after clotting and separation. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples must be stored frozen at -20°C to avoid loss of bioactive human RANTES. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (refer to "Performance characteristics" on page 4). Avoid repeated freeze-thaw cycles. Prior to assay, frozen serum or plasma should be brought to room temperature slowly and mixed gently.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 μL to 1000 μL adjustable single channel micropipettes with disposable tips
- 50 μL to 300 μL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

Precautions for use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.

- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C .
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents and samples

1. Buffer concentrate should be brought to room temperature and diluted before starting the test procedure.
2. If crystals have formed in the buffer concentrate, warm it gently until crystals have completely dissolved.

Wash buffer (1x)

1. Pour entire contents (25 mL) of the Wash Buffer Concentrate (20x) into a clean 500 mL graduated cylinder. Bring to final volume to 500 mL with glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle and store at 2°C to 25°C . Please note that Wash Buffer (1x) is stable for 30 days.

Assay buffer (1x)

Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently.

Test protocol

Note:

- Use plate immediately after removal from -20°C !
- Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!
- Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results
- Perform the washing step with at least 400 μL of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results
- Allow the washing buffer to sit in the wells for a few seconds before aspiration
- Remove covers of the standard strips carefully in order that all the lyophilised pellets remain in the wells

1. Prepare your samples before starting with the test procedure. Dilute serum samples 1:10 with Assay Buffer.

90 µL Assay Buffer + 10 µL sample

Use plasma and cell culture samples without predilution

2. Determine the number of Microwell Strips required to test the desired number of samples plus Microwell Strips for blanks and standards (colored). Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra Microwell Strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).

Table 1 Example of the arrangement of blanks, standards and samples in the microwell strips.

	1	2	3	4
A	Standard 1 2000.0 pg/mL	Standard 1 2000.0 pg/mL	Sample 1	Sample 1
B	Standard 2 1000.0 pg/mL	Standard 2 1000.0 pg/mL	Sample 2	Sample 2
C	Standard 3 500.0 pg/mL	Standard 3 500.0 pg/mL	Sample 3	Sample 3
D	Standard 4 250.0 pg/mL	Standard 4 250.0 pg/mL	Sample 4	Sample 4
E	Standard 5 125.0 pg/mL	Standard 5 125.0 pg/mL	Sample 5	Sample 5
F	Standard 6 62.5 pg/mL	Standard 6 62.5 pg/mL	Sample 6	Sample 6
G	Standard 7 31.3 pg/mL	Standard 7 31.3 pg/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

3. Add distilled water to all standard and blank wells as indicated on the label of the standard strips (A1/A2 to H1/H2).
4. Add 140 µL of distilled water to the sample wells.
5. Add 10 µL of each Sample, in duplicate, to the designated wells and mix the contents.
6. Cover with a Plate Cover and incubate at room temperature (18°C to 25°C) for 3 hours on a microplate shaker.
7. Remove Plate Cover and empty wells. Wash the microwell strips 6 times with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.

8. Pipette 100 µL of TMB Substrate Solution to all wells, including the blank wells.
9. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

The color development on the plate should be monitored and the substrate reaction stopped (see point 10. of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue color.

Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

10. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2°C to 8°C in the dark.
11. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human RANTES standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human RANTES concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating human RANTES for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human RANTES concentration.
- Samples have been diluted 1:100 (serum samples) and 1:10 (plasma samples and cell culture supernatants), thus the concentration read from the standard curve must be multiplied by the dilution factor (x 100 for serum samples and x 10 for plasma samples and cell culture supernatants).

Note: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 µL to the final volume per well. These 100 µL are composed of 90 µL of sample diluent plus 10 µL of the sample (serum samples 1:10 prediluted, plasma samples and cell culture supernatants undiluted). This is a 1:100 dilution for serum samples and a 1:10 dilution for plasma samples and cell culture supernatants.

The remaining 50 µL to give 150 µL are due to the addition of 50 µL conjugate to all wells.

90 µL sample diluent and 50 µL conjugate results in 140 µL reconstitution volume, addition of 10 µL 1:10 prediluted serum sample or undiluted plasma sample or cell culture supernatant (90 µL + 10 µL = 1:100 or 1:10 dilution).

- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human RANTES levels. Such samples require further external predilution according to expected human RANTES values with Assay Buffer (1x) in order to precisely quantitate the actual human RANTES level.
- It is suggested that each testing facility establishes a control sample of known human RANTES concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

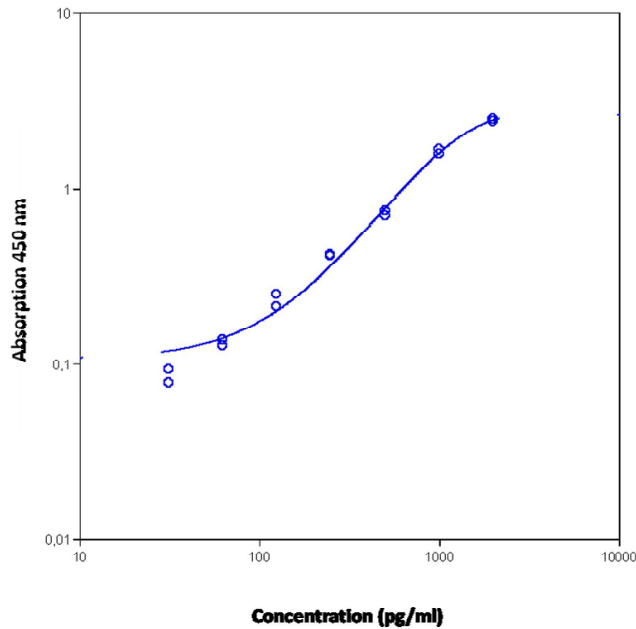


Fig. 4 Representative standard curve for human RANTES Instant ELISA. Human RANTES was diluted in serial 2-fold steps in Assay Buffer, each symbol represents the mean of 3 parallel titrations. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Table 2 Typical data using the human RANTES Instant ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	human RANTES Concentration (pg/mL)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	2000.0	2.478 2.409	2.444	1.4
2	1000.0	1.665 1.558	1.607	3.0
3	500.0	0.698 0.743	0.721	3.1
4	250.0	0.416 0.414	0.415	0.2
5	125.0	0.245 0.209	0.227	7.9
6	62.5	0.137 0.126	0.132	4.2
7	31.3	0.093 0.078	0.086	8.8
Blank	0.0	0.019 0.021	0.020	7.1

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Performance characteristics

Sensitivity

The limit of detection of human RANTES defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 4.2 pg/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human RANTES. Two standard curves were run on each plate. Data below show the mean human RANTES concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 6.9%.

Table 3 The mean human RANTES concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	Human RANTES Concentration (pg/mL)	Coefficient of Variation (%)
1	1	78,391	4.0
	2	68,759	8.0
	3	65,012	10.0
2	1	87,880	1.0
	2	76,889	9.0
	3	69,772	6.0
3	1	39,377	6.0
	2	34,468	10.0
	3	35,060	2.0
4	1	76,956	9.0
	2	68,351	9.0
	3	66,792	9.0
5	1	83,131	4.0
	2	65,719	9.0
	3	71,824	4.0
6	1	23,107	10.0
	2	19,400	3.0
	3	19,241	8.0

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments by 3 technicians. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human RANTES. Two standard curves were run on each plate. Data below (see Table 4) show the mean human RANTES concentration and the coefficient of variation calculated on

18 determinations of each sample. The calculated overall coefficient of variation was 9.9%.

Table 4 The mean human RANTES concentration and the coefficient of variation calculated on 18 determinations of each sample.

Sample	human RANTES Concentration (pg/mL)	Coefficient of Variation (%)
1	70,721	9.8
2	78,180	11.7
3	36,302	7.4
4	70,700	7.7
5	73,558	12.0
6	20,583	10.6

Spike recovery

The spike recovery was evaluated by spiking 4 levels of human RANTES into human serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments. Average recovery ranged from 80–118% with an overall mean recovery of 95.2%.

Dilution parallelism

Four serum samples with different levels of human RANTES were analyzed at serial 2-fold dilutions with 4 replicates each. The recovery ranged between 100% and 114% with an overall recovery of 105.0%.

Sample stability

Freeze-Thaw stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 3 times, and the human RANTES levels determined. There was no significant loss of human RANTES immunoreactivity between 0 and 3 freeze-thaw cycles.

Comparison of serum and plasma

There is a significantly higher level of RANTES found in serum samples than in plasma samples.

It is highly recommended to assure the uniformity of blood preparations.

Specificity

To define the specificity of this ELISA several proteins were tested for cross-reactivity. No cross-reactivity was observed.

Expected values

A panel of 21 sera from apparently healthy blood donors (male and female) was tested for human RANTES. The detected human RANTES levels ranged between 37,025 pg/mL and 10,1161 pg/mL with a mean level of 69,717 pg/mL and a standard deviation of $\pm 21,212$ pg/mL.

Reagent preparation summary

Wash buffer (1x)

Add Wash Buffer Concentrate 20 x (25 mL) to 475 mL distilled water

Assay buffer (1x)

Add Assay Buffer Concentrate 20 x (5 mL) to 95 mL distilled water

Test protocol summary

1. Predilute serum samples with Assay Buffer 1:10. Use plasma samples and cell culture supernatants undiluted.
2. Place standard strips in position A1/A2 to H1/H2.
3. Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
4. Add 140 μ L distilled water to sample wells.
5. Add 10 μ L Sample to designated wells.
6. Cover microwell strips and incubate 3 hours at room temperature (18°C to 25°C) on a microplate shaker.
7. Empty and wash microwell strips 6 times with 400 μ L Wash Buffer.
8. Add 100 μ L of TMB Substrate Solution to all wells including blank wells.
9. Incubate the microwell strips for about 10 minutes at room temperature (18°C to 25°C).
10. Add 100 μ L Stop Solution to all wells including blank wells.
11. Blank microwell reader and measure color intensity at 450 nm.

Note: Samples have been diluted 1:100 (serum samples) or 1:10 (plasma samples or cell culture supernatants), thus the concentration read from the standard curve must be multiplied by the dilution factor ($\times 100$ for serum samples, $\times 10$ for plasma samples or cell culture supernatants).

Customer and technical support

Visit thermofisher.com/support for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.



Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. All other trademarks are the property of their respective owners.

thermofisher.com/support | thermofisher.com/askaquestion

thermofisher.com

17 July 2019

ThermoFisher
SCIENTIFIC