

Human RANTES ELISA Kit

EHRNTS EHRNTS5

1365.2

Number	Description
EHRNTS	Human Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES) ELISA Kit , sufficient reagents for 96 determinations Kit Contents: Anti-Human RANTES Pre-coated 96-well Strip Plate , 1 each Lyophilized Recombinant Human RANTES Standard , 2 vials Standard Diluent , 2 × 25 ml, contains 0.1% sodium azide Biotinylated Antibody Reagent , 8 ml, contains 0.1% sodium azide 30X Wash Buffer , 50 ml Streptavidin-HRP Concentrate , 75 µl Streptavidin-HRP Dilution Buffer , 14 ml TMB Substrate Solution , 13 ml Stop Solution , 13 ml, contains 0.16 M sulfuric acid Adhesive Plate Covers , 6 each
EHRNTS5	Human RANTES ELISA Kit , sufficient reagents for 5 × 96 determinations Kit Contents: Anti-Human RANTES Pre-coated 96-well Strip Plate , 5 each Lyophilized Recombinant Human RANTES Standard , 5 vials Standard Diluent , 110 ml, contains 0.1% sodium azide Biotinylated Antibody Reagent , 35 ml, contains 0.1% sodium azide 30X Wash Buffer , 200 ml Streptavidin-HRP Concentrate , 250 µl Streptavidin-HRP Dilution Buffer , 70 ml TMB Substrate Solution , 5 × 13 ml Stop Solution , 55 ml, contains 0.16 M sulfuric acid Adhesive Plate Covers , 30 each

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

Storage: For maximum stability, store in a non-defrosting -20°C freezer and refer to the expiration date for frozen storage on the label. Alternatively, store at 2-8°C and refer to the expiration date for refrigerated storage. Once thawed, store at 4°C until the expiration date for refrigerated storage. Kit is shipped on dry ice.






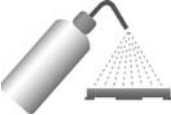


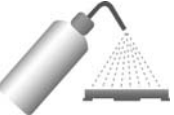



Introduction

This Thermo Scientific ELISA Kit is for measuring human RANTES in culture supernatants; EDTA plasma, sodium citrate plasma, heparin plasma; serum; and urine.

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Procedure Summary

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| 1. Add 50 µl of standards or diluted samples to each well in duplicate. | 2. Cover plate and incubate at room temperature (RT; 20-25°C) for 1 hour. | 3. Wash plate THREE times. | 4. Add 50 µl of Biotinylated Antibody Reagent to each well. |
|  |  |  |  |
| 5. Cover plate. Incubate at RT for 1 hour. | 6. Wash plate THREE times. | 7. Add 100 µl of prepared Streptavidin-HRP Solution to each well. | 8. Cover plate. Incubate at RT for 30 minutes. |
|  |  |  |  |
| 9. Wash plate THREE times. | 10. Add 100 µl of TMB Substrate to each well. Develop plate in the dark at RT for 30 minutes. | 11. Stop reaction by adding 100 µl of Stop Solution to each well. | 12. Measure absorbance on a plate reader at 450 nm minus 550 nm. Calculate results. |

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1,000 µl and plastic pipettes to deliver 5-15 ml
- A glass or plastic two-liter container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5 ml polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- 15 ml plastic tube to prepare Streptavidin-HRP Solution
- Microcentrifuge to prepare Streptavidin-HRP Solution
- A standard ELISA reader for measuring absorbance at 450 nm and 550 nm. If a 550 nm filter is not available, the absorbance can be measured at 450 nm only. Refer to the instruction manual supplied with the instrument being used.
- Graph paper or a computerized curve-fitting statistical software package

Precautions

- **All samples and reagents must be at room temperature (20-25°C) before use in the ELISA.**
- Review the instructions carefully and verify all components against the Kit Contents list (page 1) before beginning.
- Do not use a water bath to thaw samples. Thaw samples at room temperature.
- When preparing standard curve and sample dilution in culture medium, use the same medium used to culture the cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture cells, then use RPMI with 10% FCS to dilute the standard and samples. Do NOT use RPMI without serum supplement.
- To avoid cross-contamination, always use a new disposable reagent reservoir. Also use new disposable pipette tips for each transfer and a new adhesive plate cover for each incubation step.
- Once reagents have been added to the plate, take care NOT to let plate DRY at any time during the assay.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Do not mix reagents from different kit lots. Discard unused kit components.
- Do not use glass pipettes to measure TMB Substrate. Take care not to contaminate the solution. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Some components of this kit contain sodium azide. Please dispose of reagents according to local regulations.

Additional Precautions for the 5-plate kit

- Dispense only reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.
- Use only one bottle of the TMB Substrate per 96-well plate. Do not combine leftover substrate with that reserved for other plates.
- Equilibrate to room temperature only the reagent volumes required for the number of plates being used.
- Use only one vial of standard per 96-well plate.

Sample Preparation

Sample Handling

- Serum; EDTA, heparin and sodium citrate plasma; urine or culture supernatants may be tested in this ELISA.
- For **serum or plasma**, 50 µl per well of sample, diluted 1:100, are required. For **urine or culture supernatant**, 50 µl per well of sample are required.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles when storing samples.
- Test samples and standards must be assayed in duplicate each time the ELISA is performed.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use heated water baths to thaw or warm samples. Mix samples by gently inverting tubes.
- If samples are clotted, grossly hemolyzed, lipemic or microbially contaminated, or if there is any question about the integrity of a sample, make a note on the template and interpret results with caution.

Sample Dilution

- **Culture supernatant and urine samples** may be assayed neat (undiluted) in the ELISA.

Note: We have observed 4-50 ng/ml of RANTES in culture supernatants from stimulated human cells. If the human RANTES concentration of a culture supernatant sample possibly exceeds the highest point of the standard curve (i.e., 2,000 pg/ml), multiple sample dilutions may be required.

- **Serum and plasma samples** must be diluted 1:100 in Standard Diluent before assaying by adding 5 µl of serum or plasma to 495 µl of Standard Diluent. Mix sample well. If the human RANTES concentration of a test sample diluted 1:100 possibly exceeds the highest point of the standard curve (i.e., 2,000 pg/ml), prepare one or more additional five-fold dilutions. For example, a further dilution of five-fold is prepared by adding 50 µl of diluted sample to 200 µl of Standard Diluent. Mix sample thoroughly between dilutions before assaying.

Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout these instructions.

Note: When using the 5-plate kit, only one standard per plate is supplied. Therefore, partial plates cannot be used.

Wash Buffer

1. Label a clean glass or plastic two-liter container “Wash Buffer.” The 30X Wash Buffer may have a cloudy appearance.
2. Add the entire contents of the 30X Wash Buffer bottle (50 ml) to the two-liter container and dilute to a final volume of 1.5 liters with ultrapure water. Mix thoroughly.

(PP) When using partial plates, store the reconstituted Wash Buffer at 2-8°C.

Note: Wash Buffer must be at room temperature before use in the assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

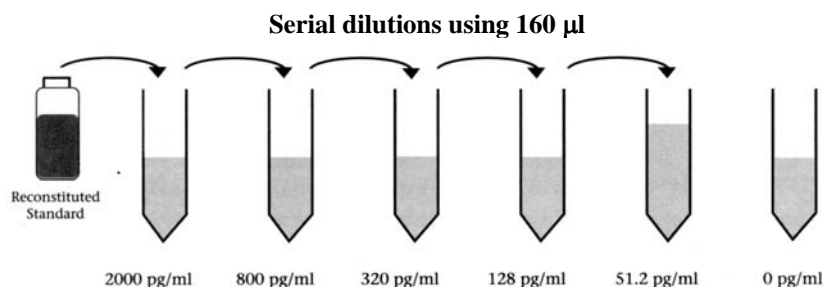
Note: If using the 5-plate kit, add 30 ml Wash Buffer to 870 ml water for each plate used.

Standards

- **(PP)** Reconstitute and use one vial of the lyophilized standard per partial plate.
 - Prepare standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.
1. When testing **culture supernatant samples**, reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use the sample culture medium to prepare standard curve serial dilutions.

When testing **serum, plasma or urine samples**, reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use the Standard Diluent provided to prepare standard curve serial dilutions.

2. Label six tubes, one for each standard curve point: 2,000 pg/ml, 800 pg/ml, 320 pg/ml, 128 pg/ml, 51.2 pg/ml, and 0 pg/ml, then prepare 1:2.5 serial dilutions for the standard curve as follows:
3. Pipette 240 µl of appropriate diluent into each tube.
4. Pipette 160 µl of the reconstituted standard into the first tube (i.e., 2,000 pg/ml) and mix.
5. Pipette 160 µl of this dilution into the second tube (i.e., 800 pg/ml) and mix.
6. Repeat the serial dilutions (using 160 µl) three more times to complete the standard curve points.



Assay Procedure

A. Sample Incubation

- **(PP)** Determine the number of strips required. Leave these strips in the plate frame. Tightly seal remaining unused strips in the provided foil pouch with desiccant and store at 2-8°C. After completing assay, retain plate frame for second partial plate. When using the second partial plate, place strips securely in the plate frame.
 - Use the Data Template provided to record the locations of the zero standard (blank or negative control), human RANTES standards and test samples. Perform five standard points and one blank in duplicate with each series of unknown samples.
1. Add 50 µl of reconstituted standards or test samples in duplicate to each well. Mix well by gently tapping the plate several times.

Note: Serum and plasma samples should have been diluted 1:100 before use in this assay. If the human RANTES concentration in any sample possibly exceeds the highest point on the standard curve, 2,000 pg/ml, see Sample Preparation–Sample Dilution section.

2. Add 50 µl of Standard Diluent to all wells that do not contain standards or samples.
3. Carefully cover plate with a new adhesive plate cover. Ensure all edges and strips are sealed tightly by running your thumb over edges and down each strip. Incubate for one (1) hour at room temperature, 20-25°C.
4. Carefully remove adhesive plate cover. Wash plate **THREE** times with Wash Buffer as described in the Plate Washing section (section B).

B. Plate Washing

1. Gently squeeze the long sides of plate frame before washing to ensure all strips securely remain in the frame.
2. Empty plate contents. Use a squirt bottle to vigorously fill each well completely with Wash Buffer, then empty plate contents. Repeat procedure two additional times for a total of **THREE** washes. Blot plate onto paper towels or other absorbent material.

Note: For automated washing, aspirate all wells and wash **THREE** times with Wash Buffer, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

C. Biotinylated Antibody Reagent Incubation

- If using a multichannel pipettor, **use a new reagent reservoir and pipette tips** when adding the Biotinylated Antibody Reagent. Remove from the vial only the amount required for the number of strips being used. Take care not to touch the samples in wells with the pipette tip when adding the Biotinylated Antibody Reagent.
1. Add 50 µl of Biotinylated Antibody Reagent to each well containing sample or standard.
 2. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for one (1) hour at room temperature, 20-25°C.
 3. Carefully remove the adhesive plate cover, discard plate contents and wash **THREE** times as described in the Plate Washing section (section B).

D. Streptavidin-HRP Solution Preparation and Incubation

- Prepare Streptavidin-HRP Solution **immediately before use**. Do not prepare more solution than required. Do not store prepared Streptavidin-HRP Solution.
 - Use a 15 ml plastic tube to prepare Streptavidin-HRP Solution.
 - If using a multichannel pipettor, **use new reagent reservoir and pipette tips** when adding the prepared Streptavidin-HRP Solution.
1. Briefly centrifuge the Streptavidin-HRP Concentrate to force entire vial contents to the bottom.

2. **(PP)** Use only the Streptavidin-HRP Solution amount required for the number of strips being used. For each strip, mix 2.5 µl of Streptavidin-HRP Concentrate with 1 ml of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.

For one complete 96-well plate, add 30 µl of Streptavidin-HRP Concentrate to 12 ml of Streptavidin-HRP Dilution Buffer and mix gently.

3. Add 100 µl of prepared Streptavidin-HRP Solution to each well.
4. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.
5. Carefully remove the adhesive plate cover, discard plate contents and wash THREE times as described in the Plate Washing section.

E. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding TMB Substrate and Stop Solution.
 - Dispense from bottle ONLY amount required, 100 µl per well, for the number of wells being used. Do not use a glass pipette to measure the TMB Substrate.
 - **(PP)** Do not combine leftover substrate with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate.
1. Pipette 100 µl of TMB Substrate into each well.
 2. Allow color reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate with aluminum foil or a plate sealer. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
 3. After 30 minutes, stop the reaction by adding 100 µl of Stop Solution to each well.

F. Absorbance Measurement

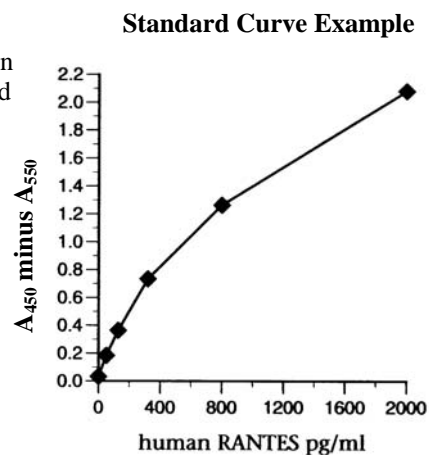
Note: Evaluate the plate within 30 minutes of stopping the reaction.

Measure the absorbance on an ELISA plate reader set at 450 nm and 550 nm. Subtract 550 nm values from 450 nm values to correct for optical imperfections in the microplate. If an absorbance at 550 nm is not available, measure the absorbance at 450 nm only.

Note: When the 550 nm measurement is omitted, absorbance values will be higher.

G. Calculation of Results

- The standard curve is used to determine human RANTES amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding human RANTES concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the human RANTES amount in each sample by interpolating from the absorbance value (Y axis) to human RANTES concentration (X axis) using the standard curve. For **culture supernatant and urine samples** that are assayed neat, the pg/ml of RANTES can be interpolated directly from the standard curve. **Serum and plasma samples** should have been diluted 1:100 before performing the assay. For these samples, multiply the value interpolated from the standard curve by 100 to calculate the pg/ml of RANTES.
- If the test sample was further diluted, multiply the interpolated value obtained from the standard curve by the total dilution factor to calculate pg/ml of human RANTES in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Duplicate values that differ from the mean by greater than 10% should be considered suspect and repeated.



Performance Characteristics

Sensitivity: 2 pg/ml

The sensitivity or Lower Limit of Detection (LLD)¹ is determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

Assay Range: 51.2-2,000 pg/ml

Suggest standard curve points are 2,000, 800, 320, 128, 51.2, and 0 pg/ml.

Reproducibility:

Intra-Assay CV: < 10%

Inter-Assay CV: < 10%

Specificity: This ELISA is specific for the measurement of natural and recombinant human RANTES. This ELISA does not cross-react with the following chemokines and cytokines: rat RANTES, human Eotaxin, GRO α , GRO β , MIP-1 α , MIP-1 β , MCP-1, MCP-2, MCP-3, MCP-4, G-CSF, GM-CSF, IFN α , IFN γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, TNF α or TNF β .

Calibration:

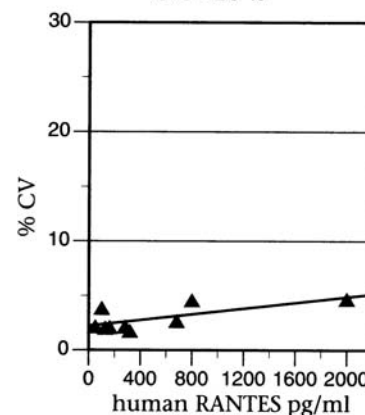
The standard in this ELISA is calibrated to the NIBSC recombinant RANTES standard 95/520.

One (1) pg of internal standard = 1 pg of NIBSC standard = 0.1 NIBSC units.

Precision:

The intra-assay coefficient of variation is plotted against RANTES concentration (pg/ml). The points represent samples evaluated in replicates of four in four different kit lots.

hRANTES ELISA Precision Profile
CVs < 10 %



Expected Values:

Serum, plasma, and urine samples collected from apparently healthy human donors are evaluated in this assay. The levels of human RANTES found in each sample type are reported below:

<u>Sample Type</u>	<u>Average</u>	<u>Range</u>
Serum (n=5)	42.3 ng/ml	8.9-106.1 ng/ml
EDTA Plasma (n=27)	53.4 ng/ml	4.4-146.7 ng/ml
Heparin Plasma (n=7)	11.7 ng/ml	4.5-20.9 ng/ml
Sodium Citrate Plasma (n=7)	10.8 ng/ml	2.7-29.4 ng/ml
Urine (n=8)	9.4 pg/ml	3.1-27.6 pg/ml

Recovery:

Recovery in serum and in plasma collected with various anticoagulants is determined by spiking 1,000 pg/ml recombinant human RANTES into matched serum and plasma samples collected from five apparently healthy individuals and a Standard Diluent control buffer. Mean recoveries are as follows:

<u>Sample Type</u>	<u>Average Recovery</u>	<u>Range</u>
Serum (n=5)	81%	79-88%
EDTA Plasma (n=5)	91%	86-95%
Heparin Plasma (n=5)	96%	92-105%
Sodium Citrate Plasma (n=5)	96%	93-104%

Recovery across the range of the standard curve is determined by spiking various levels of recombinant human RANTES into serum, plasma and urine samples collected from apparently healthy individuals, and a Standard Diluent control buffer. Mean recoveries are as follows:

<u>Spike Level:</u>	<u>500 pg/ml</u>	<u>1,000 pg/ml</u>	<u>1,500 pg/ml</u>
Serum (n=5)	126%	86%	85%
EDTA Plasma (n=5)	122%	87%	90%
<u>Spike Level:</u>	<u>1,000 pg/ml</u>		
Urine (n=8)	81%		

Reference

1. *Immunoassay: A Practical Guide*, Chan and Perlstein, Eds., 1987. Academic Press: New York, p71.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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Data Templates

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
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