Human RANTES ELISA Kit

EHRNTS EHRNTS2 EHRNTS5

Number	Description
EHRNTS	**Human Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES) ELISA Kit,** sufficient reagents for 96 determinations
EHRNTS2	**Human RANTES ELISA Kit,** sufficient reagents for 2 × 96 determinations
EHRNTS5	**Human RANTES ELISA Kit,** sufficient reagents for 5 × 96 determinations

Kit Contents	EHRNTS	EHRNTS2	EHRNTS5
Antibody Coated Plate, 96-well plate	1 each	2 each	5 each
Lyophilized Recombinant Human RANTES Standard	2 vials	4 vials	10 vials
Standard Diluent, contains 0.1% NaN_3	2 × 25mL	4 × 25mL	10 × 25mL
Biotinylated Antibody Reagent, contains NaN_3	8mL	2 × 8mL	5 × 8mL
30X Wash Buffer	50mL	2 × 50mL	5 × 50mL
Streptavidin-HRP Concentrate	75µL	2 × 75µL	5 × 75µL
Streptavidin-HRP Dilution Buffer	14mL	2 × 14mL	5 × 14mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 0.16M sulfuric acid	13mL	2 × 13mL	5 × 13mL
Adhesive plate covers	6 each	12 each	30 each

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

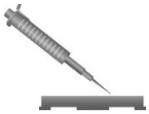
Storage: Upon receipt, store the kit at 2-8ºC.

Table of Contents

Introduction

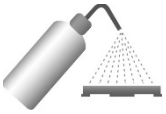
The Thermo Scientific™ Human RANTES ELISA Kit is for measuring human RANTES in culture supernatants; EDTA plasma, sodium citrate plasma, heparin plasma; serum; and urine.

Procedure Summary

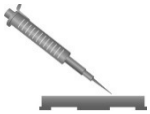


1. Add 50µL of standards or diluted samples to each well in duplicate.

2. Cover plate and incubate at room temperature (RT; 20-25°C) for 1 hour.

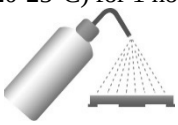


3. Wash plate THREE times.



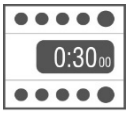
4. Add 50µL of Biotinylated Antibody Reagent to each well.

5. Cover plate. Incubate at RT for 1 hour.

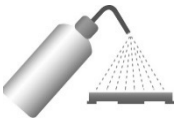


6. Wash plate THREE times.

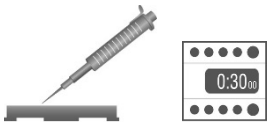
7. Add 100µL of prepared Streptavidin-HRP Solution to each well.



8. Cover plate. Incubate at RT for 30 minutes.



9. Wash plate THREE times.



10. Add 100µL of TMB Substrate to each well. Develop plate in the dark at RT for 30 minutes.

11. Stop reaction by adding 100µL of Stop Solution to each well.

12. Measure absorbance on a plate reader at 450nm minus 550nm. Calculate results.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000µL and plastic pipettes to deliver 5-15mL
- A glass or plastic two-liter container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5mL polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- 15mL plastic tube to prepare Streptavidin-HRP Solution
- Microcentrifuge to prepare Streptavidin-HRP Solution
- A standard ELISA reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance can be measured at 450nm 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 2-plate and 5-plate Kits

- Dispense, pool and equilibrate to room temperature only reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.

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,** 50µL per well of sample, diluted 1:100, are required. For **urine or culture supernatant,** 50µL 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 4-50ng/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., 2000pg/mL), multiple sample dilutions may be required.

- **Serum and plasma samples** must be diluted 1:100 in Standard Diluent before assaying by adding 5µL of serum or plasma to 495µL 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., 2,000pg/mL), prepare one or more additional five-fold dilutions. For example, a further dilution of five-fold is prepared by adding 50µL of diluted sample to 200µL 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.

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 (50mL) to the 2L container and dilute to a final volume of 1.5L 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.

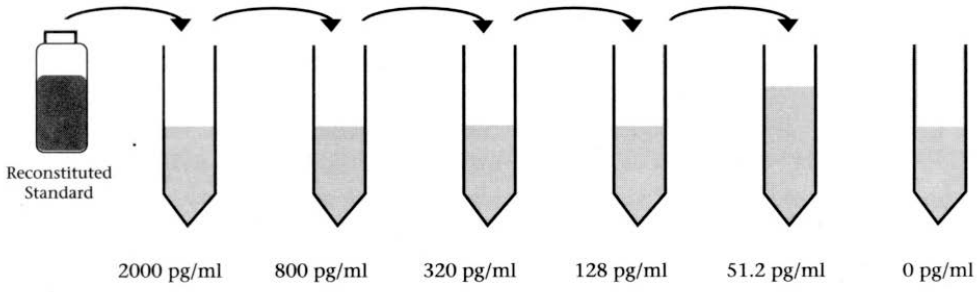
Standards

- **(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: 2000pg/mL, 800pg/mL, 320pg/mL, 128pg/mL, 51.2pg/mL, and 0pg/mL, then prepare 1:2.5 serial dilutions for the standard curve as follows:
3. Pipette 240µL of appropriate diluent into each tube.
4. Pipette 160µL of the reconstituted standard into the first tube (i.e., 2000pg/mL) and mix.
5. Pipette 160µL of this dilution into the second tube (i.e., 800pg/mL) and mix.
6. Repeat the serial dilutions (using 160µL) three more times to complete the standard curve points.

Serial dilutions using 160 µL



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 50µL 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, 2000pg/mL, see Sample Preparation–Sample Dilution Section.

2. Add 50µL 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 50µL 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 15mL 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 2.5µL of Streptavidin-HRP Concentrate with 1mL of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.

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

3. Add 100µL 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, 100µL 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 100µL 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 100µL of Stop Solution to each well.

F. Absorbance Measurement

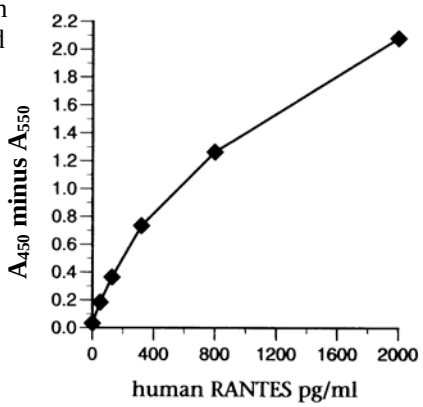
Note: Evaluate the plate within 30 minutes of stopping the reaction.

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

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

G. Calculation of Results

Standard Curve Example



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

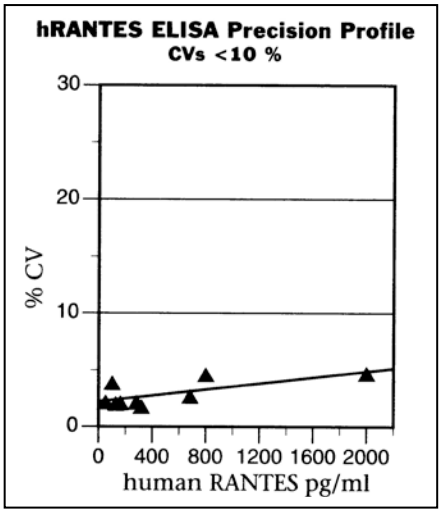
Reproducibility:

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, GROα, GROβ, MIP-1α, MIP-1β, MCP-1, MCP-2, MCP-3, MCP-4, G-CSF, GM-CSF, IFNα, IFNγ, IL-1α, IL-1β, 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, TNFα or TNFβ.

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.



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:

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

Recovery:
Recovery in serum and in plasma collected with various anticoagulants is determined by spiking 1000pg/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:

Sample Type	Average Recovery	Range
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:

Spike Level:	500pg/mL	1,000pg/mL	1,500pg/mL
Serum (n=5)	126%	86%	85%
EDTA Plasma (n=5)	122%	87%	90%

Spike Level:	1,000pg/mL
Urine (n=8)	81%

Cited Reference

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

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
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Data Templates

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