Performance characteristics, continued

Intra-assay precision

Samples of known rat RANTES concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	253.3	475.6	2028.0
SD	10.5	15.6	49.1
%CV	4.1	3.3	2.4

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	268.7	511.9	2027.8
SD	18.6	36.0	49.2
%CV	6.9	7.0	2.4

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

The recovery of rat RANTES added to rat serum averaged 80%. The recovery of rat RANTES added to tissue culture medium containing 1% fetal calf serum averaged 95%, while the recovery of rat RANTES added to tissue culture medium containing 10% fetal calf serum averaged 89%.

Linearity of dilution

Rat serum containing 1,985 pg/ml of measured rat RANTES was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.999.

Expected values

A limited number (n = 8) of rat sera were assayed with the Rat RANTES ELISA kit. The mean value obtained was 5,610 pg/mL (range: 4,140 – 7,830 pg/mL).

Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

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 found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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

For support visit **thermofisher.com/techresources** or contact **techsupport@lifetech.com**.

thermofisher.com/lifescience

08 September 2015

invitrogen

Rat RANTES ELISA Kit

Catalog no. KRC1031

Quantity: 96 tests

Pub. No. MAN0004876

Rev 1.0

Description

The Rat RANTES ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of rat RANTES in serum, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant rat RANTES.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	Cat. No. KRC1031 96 tests
Rat RANTES Antibody Coated Wells. 96 well plate.	1 plate
Rat RANTES Biotin Conjugate. Contains 0.1% sodium azide.	11 mL
Rat RANTES Standard, recombinant Rat RANTES. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Wash Buffer Concentrate (25X).	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	25 mL
Streptavidin-HRP (100X). Contains 3.3 mM thymol.	0.125 mL
Streptavidin-HRP Diluent. Contains 3.3 mM thymol.	25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL
Stop Solution.	25 mL
Adhesive Plate Covers.	3

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com/techresources** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

For Research Use Only. Not for use in diagnostic procedures.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

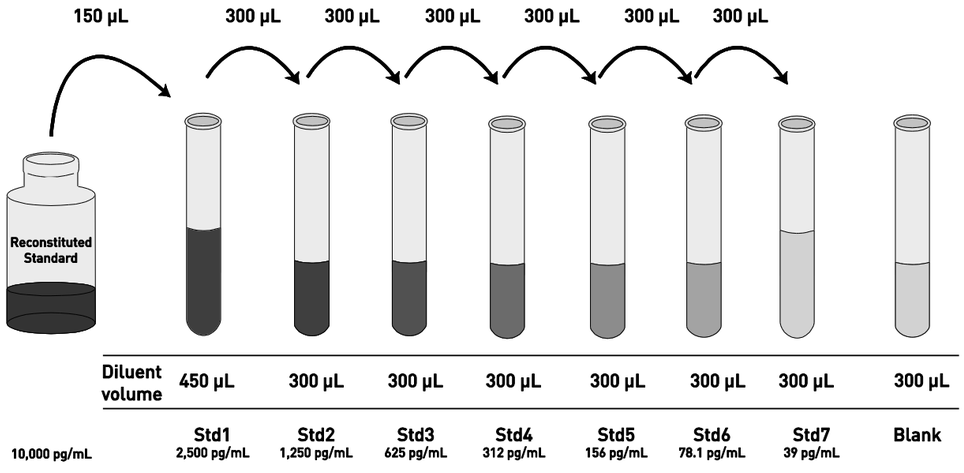
Dilute samples

- Dilute **serum** samples 20-fold in Standard Diluent Buffer.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Rat RANTES Standard to 10,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 10,000 pg/mL Rat RANTES. **Use the standard within 1 hour of reconstitution**.
2. Add 150 µL Reconstituted Standard to one tube containing 450 µL Standard Diluent Buffer and label as 2,500 pg/mL Rat RANTES.
3. Add 300 µL Standard Diluent Buffer to each of 6 tubes labeled as follows: 1,250; 625; 312; 156; 78; and 39 pg/mL Rat RANTES.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Discard remaining reconstituted standard. Return Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

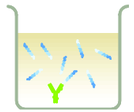
1. Allow Streptavidin-HRP (100X) to reach room temperature and mix gently.
2. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
3. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 4 hours.**

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.

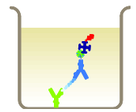


Bind antigen

1. Add 100 µL Standard Diluent Buffer to zero standard wells. Wells for chromogen blank should be left empty.
2. For the standard curve, add 100 µL of standards to the appropriate wells. For serum samples, add 100 µL of diluted samples (see Dilute samples) to the wells. For buffered solutions or cell culture samples, add 50 µL of sample followed by 50 µL of Standard Diluent Buffer.
3. Tap the side of the plate to mix. Cover the plate with the plate cover and incubate for 2 hours at 37°C.
4. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add biotin conjugate

5. Add 100 µL Rat RANTES Biotin Conjugate solution into each well except the chromogen blanks.
6. Cover the plate with plate cover and incubate for 1 hour at 37°C.
7. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.



Add Streptavidin-HRP

8. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
9. Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
10. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

Add chromogen

11. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
12. Incubate for 30 minutes at room temperature **in the dark**.

    Note: TMB should not touch aluminum foil or other metals.

Add stop solution

13. Add 100 µL of Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0-2,500 pg/mL rat RANTES.

Standard Rat RANTES (pg/mL)	Optical Density (450 nm)
2,500	2.42
1,250	1.27
625	0.62
312	0.32
156	0.20
78.1	0.12
39	0.09
0	0.07

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Rt RANTES kit. The following substances were tested and found to have no cross-reactivity: **Rat** IL-1β, IL-4, IFN-γ, MIP-2, TNF-α; **Mouse** IL-2, IL-3, IL-4, IL-6, IL-10, TNF-α; **Human** IL-1β, IL-2, IL-8, IL-10, IL-15, GM-CSF, IFN-γ, RANTES, TNF-α.

Sensitivity

The minimum detectable dose of rat RANTES is <20 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

3