novex

life technologies

Mouse RANTES ELISA Kit

Catalog nos. KMC1031 Quantity: 96 tests

Pub. No. MAN0004089 Rev 1.0

Description

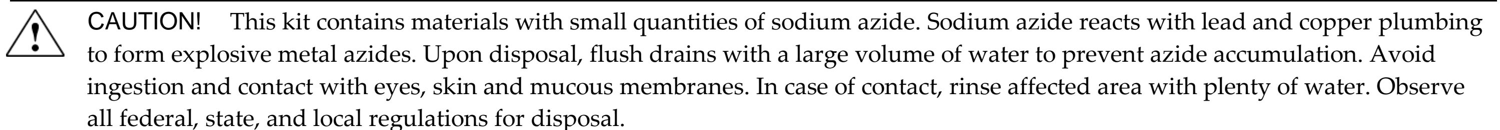
The Mouse RANTES (Ms RANTES) ELISA Kit is a solid-phase sandwich Enzyme Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of Ms RANTES in serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Ms RANTES.

RANTES (Regulated Upon Activation, Normal T-Cell Expressed and Secreted) is a member of the C-C chemokine family (beta-chemokine family). The murine RANTES homolog, is approximately 85% homologous with human RANTES. RANTES is expressed by T cells, epithelial cells, monocytes, fibroblasts, and mesangial cells. Platelets and eosinophils release RANTES from intracellular storage granules upon activation.

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	96 Test Kit
Ms RANTES Antibody Coated Wells. 96 well plate.	1 plate
Ms RANTES Biotin Conjugate. Contains 0.1% sodium azide.	6 mL
Ms RANTES Standard. Lyophilized. 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 **www.lifetechnologies.com/manuals** 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 20 mL of Wash Buffer Concentrate (20X) with 380 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.

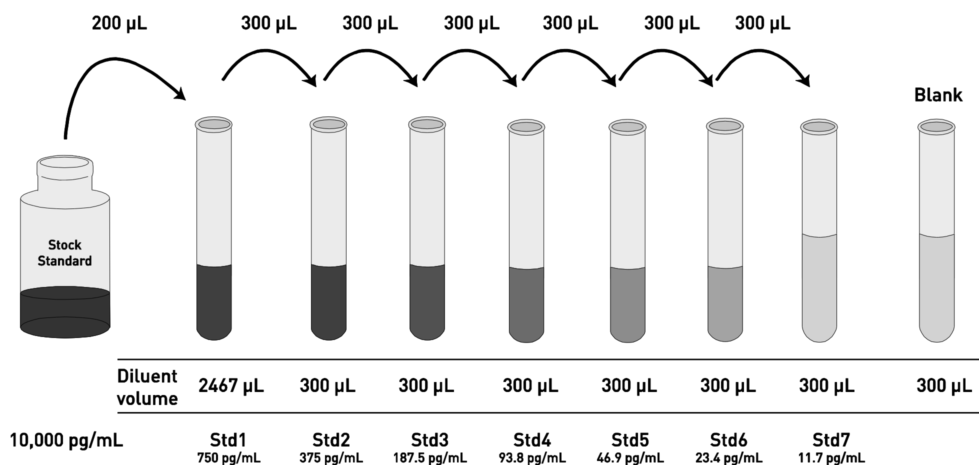
Dilute samples

- Dilute serum and plasma samples 2-fold in Standard Diluent Buffer.
- Buffered solutions, cell culture samples, and controls may be assayed without dilution.
- 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 Ms 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 Ms RANTES.
2. Add 200 µL Reconstituted Standard to one tube containing 2467 µL Standard Diluent Buffer and label as 750 pg/mL Ms RANTES.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 375, 187.5, 93.8, 46.9, 23.4, 11.7 and 0 pg/mL Ms RANTES.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare Streptavidin-HRP solution

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

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

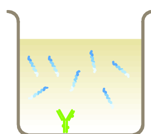
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X)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

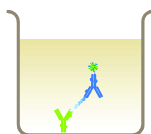
IMPORTANT! Perform a standard curve with each assay.

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.



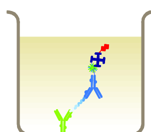
Bind antigen

1. Add 100 µL of standards, **tissue culture supernant**, or controls into the appropriate wells.
2. Add 50 µL of Standard Diluent Buffer to sample wells, followed by 50 µL of **serum/plasma** samples (see page 2) in the appropriate wells.
3. Cover the plate with plate cover and incubate for 2 hours at room temperature.
4. Thoroughly aspirate the solution and wash wells 6 times with 1X Wash Buffer.



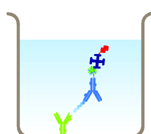
Add detector antibody

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



Add Streptavidin-HRP

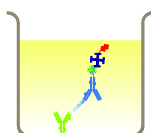
8. Add 100 µL Streptavidin-HRP (see page 2) into each well except the chromogen blanks.
9. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
10. Thoroughly aspirate the solution from the wells and wash wells 6 times with 1X Wash Buffer.



Add chromogen

11. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
12. Cover the plate with plate cover and 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 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

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Mouse RANTES kit. The following substances were tested and found to have no cross–reactivity: **Human** IL-12, IL-13, IL-15, IL-17, and RANTES; **Mouse** IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, GM-CSF, IFN-γ, TNF-α, and eotaxin; **Rat** IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, GM-CSF, IFN-γ, MIP-2, and TNF-α; The cross–reactivity with the recombinant rat RANTES was measured to be >80%.

Sensitivity

The minimum detectable concentration of Ms RANTES is <5 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Performance characteristics, continued

Intra-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	143.1	274.5	764.6
SD	1.8	7.4	20.8
%CV	1.24	2.7	2.7

SD = Standard Deviation; CV = Coefficient of Variation

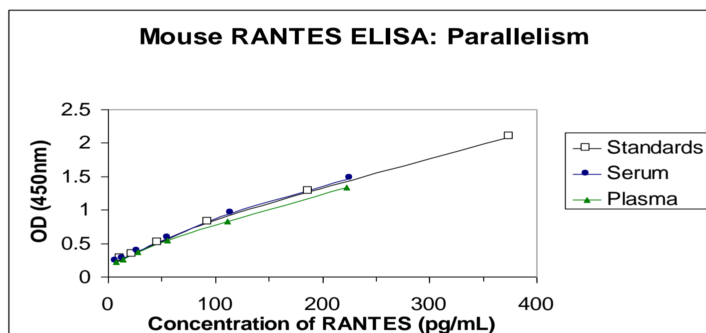
Recovery

Below are the average recoveries when adding Ms RANTES to the following samples.

Sample	% Recovery
Serum	89
Plasma (citrate and EDTA)	92
Culture medium with 1% and 10% calf serum	117
Culture medium with 10% fetal bovine serum	108

Parallelism

Mouse serum and plasma containing natural RANTES were serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the mouse RANTES standard curve. Parallelism is demonstrated by the figure below and indicated that the standard accurately reflects the mouse RANTES content in samples.



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)	127.4	274.4	753.4
SD	12.8	6.8	25.4
%CV	10.0	2.4	3.4

SD = Standard Deviation; CV = Coefficient of Variation

Linearity of dilution

Mouse serum, plasma or tissue culture medium spiked with 700 pg/mL of measured Ms RANTES were 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 0.960, 0.968, and 0.970, respectively.

Expected values

A limited number (n=23) of mouse serum and plasma (citrate and EDTA) samples were assayed with the Ms RANTES kit. The mean value obtained was 124 pg/mL (range: 5–250 pg/mL).

Standard curve (example)

The following data were obtained for the various standards over the range of 0–750 pg/mL Ms RANTES.

Standard Ms RANTES (pg/mL)	Optical Density (450)
750	2.77
375	1.82
187.5	1.08
93.8	0.68
46.9	0.45
23.4	0.33
11.7	0.28
0	0.20

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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

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