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Human RANTES ELISA Kit

Catalog No: CDK048A **Size:** 1 x 96 tests
CDK048B 2 x 96 tests

Specificity:	Native and recombinant human RANTES (CCL5)
Sensitivity:	1.2 pg/ml
Range:	6.25 – 250 pg/ml
Sample Type:	Serum, plasma, cell culture supernatant
Cross-Reactivity:	No cross reactivity with other human cytokines
Incubation:	1 hour 45 minutes
Sample Size:	100 µl

1. INTRODUCTION

The Cell Sciences® Human RANTES ELISA kit is a solid phase sandwich ELISA for the in-vitro qualitative and quantitative determination of Regulated upon Activation, Normal T Cell Expressed and Secreted (RANTES), also known as C-C chemokine ligand 5 (CCL5), in supernatants, buffered solutions or serum and plasma samples. This assay will recognize both natural and recombinant human RANTES.

Principle of the ELISA method
A capture antibody highly specific for the analyte has been coated to the wells of the microtiter strip plate during manufacture. Binding of the analyte in samples and known standards to the capture antibodies, and subsequent binding of the biotinylated detection antibody to the analyte, is completed during the same incubation period. Any excess unbound analyte and detection antibody is then removed. The HRP conjugate solution is then added to every well, including the zero wells. Following incubation, excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue-colored complex with the conjugate. The color development is then stopped by the addition of acid, turning the resulting final product yellow. The intensity of the produced colored complex is directly proportional to the concentration of analyte present in the samples and standards. The absorbance of the color complex is measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of analyte in any sample tested.

2. BACKGROUND

CCL5, also known as Regulated upon Activation, Normal T Cell Expressed and Secreted (RANTES), is a member of the CC family of chemokine (Chemotactic cytokines that control the migration of immune cells in tissue). The mature form of the protein consists of 68 amino acids with a molecular mass of approximately 8 kDa.

Mainly expressed by T-cells and monocytes and abundantly by epithelial cells, fibroblasts, and thrombocytes, it plays an active role in recruiting leucocytes into inflammatory sites through receptors CCR1, CCR3, CCR4 and CCR5 and inducing their proliferation and activation.

Described as the major HIV-1 co-receptor, it inhibits virus entry and replication within cells at low concentration. The CCR5-CCL5 blockade maybe thus an effective strategy for HIV prevention.

RANTES is involved in transplantations (level is higher during rejection of renal transplant) and plays an important role in various human disorders, such as atherosclerosis, COVID-19, SARS, atopic dermatitis, asthma,

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glomerulonephritis, alcohol liver disease, acute liver failure and viral hepatitis. Increased levels of RANTES was discovered in many cancers, hepatocellular carcinoma, stomach, prostate and pancreatic cancer).

3. REAGENTS PROVIDED: RECONSTITUTION & STORAGE GUIDELINES

REAGENTS (Store at 2-8°C)	1 x 96 wells	2 x 96 wells	RECONSTITUTION & STORAGE (see Section 8 – Assay Preparation)
P. Anti-RANTES coated plate	1	2	Ready-to-use (pre-coated 96-well strips)
Q. Plastic plate cover	2	4	n/a
A. RANTES Standard (250 pg/ml)	2 vials	4 vials	**Reconstitute as directed on vial** (see Assay preparation, section 8)
B. Standard Diluent	1 vial (15 ml)	1 vial (25 ml)	10x concentrate, dilute in distilled water
C. Biotinylated anti-RANTES	1 vial (0.4 ml)	2 vials (0.4 ml)	**Dilute in Biotinylated Antibody Diluent.** (see Assay preparation, section 8)
D. Biotinylated Antibody Diluent	1 vial (7 ml)	1 vial (13 ml)	Ready-to-use.
E. Streptavidin-HRP	2 vials (5 µl)	4 vials (5 µl)	**Add 0.5ml of HRP Diluent prior to use.** (see Assay preparation, section 8).
F. Streptavidin-HRP Diluent	1 vial (12 ml)	1 vial (23 ml)	Ready-to-use.
G. Wash Buffer	1 vial (10 ml)	2 vials (10 ml)	200x concentrate, dilute in distilled water. (see Assay preparation, section 8)
H. TMB Substrate	1 vial (11 ml)	1 vial (24 ml)	Ready-to-use.
I. H_2SO_4 Stop Reagent	1 vial (11 ml)	2 vials (11 ml)	Ready-to-use.

4. MATERIALS/ REAGENTS REQUIRED BUT NOT PROVIDED

- Microtiter plate reader with appropriate filters (450nm required with optional 620nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000 µl adjustable single-channel micropipettes with disposable tips
- 50-300 ml multi-channel micropipette with disposable tips
- Multi-channel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, sterile, if possible.

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5. STORAGE INSTRUCTIONS

Store the kit reagents between 2 and 8°C. Immediately after use, remaining reagents should be returned to cold storage (2-8°C). Expiration date of the kit and reagents is stated on box front labels. The expiration date 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 during handling.

Wash Buffer 1X: Once prepared, store at 2-8°C for up to 1 week.

Standard Diluent Buffer 1X: Once prepared, store at 2-8°C for up to 1 week.

Reconstituted Standard: Once prepared use immediately and do not store.

Diluted Biotinylated Anti-RANTES: Once prepared use immediately and do not store.

Diluted Streptavidin-HRP: Once prepared use immediately and do not store.

6. SPECIMEN COLLECTION, PROCESSING & STORAGE

Cell culture supernatants, human serum, plasma, or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Cell culture supernatants: Remove particulates and aggregates by centrifugation at 1000 x g for 10 min.

Serum: Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

Plasma: EDTA, citrate and heparin plasma can be assayed. Centrifuge samples at 1000 x g for 30 min to remove particulates. Harvest plasma.

Storage: If not analyzed shortly after collection, samples should be aliquoted (250-500µl) to avoid repeated freeze-thaw cycles and stored frozen at –80°C. Avoid multiple freeze-thaw cycles of frozen specimens.

Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before use. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

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7. SAFETY AND PRECAUTIONS FOR USE

- FOR RESEARCH ONLY. Not for diagnostic use.
- Handling of reagents, serum, or plasma specimens should be in accordance with local safety procedures (e.g., CDC/NIH Health manual: “Biosafety in Microbiological and Biomedical Laboratories" 1984.)
- Laboratory gloves should be worn at all times.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated or frozen, as indicated.
- All reagents should be warmed to room temperature before use.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a new pipette tip for each reagent, standard, or specimen to avoid cross contamination.
- Use a clean plastic container to prepare the washing solution.
- All residual wash liquid must be drained from the wells by efficient aspiration, or by decantation, followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- When pipetting reagents, maintain a consistent order of addition from well-to-well to ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- **Warning**: TMB and H_2SO_4 are hazardous: avoid direct contact. In case of contact, wash thoroughly with water. Dispose of properly.
- For the dispensing of TMB and H_2SO_4, avoid pipettes with metal parts and all contact with metal.
- For TMB, if a dark blue color develops within a few minutes after preparation, the TMB solution has been contaminated and must be discarded.
- TMB solution is light sensitive. Avoid prolonged exposure to light.
- Read absorbances within 1 hour after completion of the assay.

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8. ASSAY PREPARATION

Bring all reagents to room temperature before use.

Assay Design

Determine the number of microwell strips required to test the desired number of samples, plus the number of wells needed for blanks and standards. Each sample, standard and blank should be tested **in duplicate**. Remove sufficient microwell strips for testing from the pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8°C storage.

Example Plate Layout (for a 6-point standard curve)**:**

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

All remaining empty wells can be used to test samples in duplicate.

Preparation of Wash Buffer

If crystals have formed in the concentrate Wash Buffer, warm it gently until complete dissolution.

Dilute the (200X) Wash Buffer Concentrate 200-fold with distilled water to yield a 1X working solution. Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder. Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle. *Store 1X Wash Buffer at 2°-8°C for up to 1 week.*

Preparation of Standard Diluent Buffer 1X

If crystals have formed in the concentrate Standard Diluent, warm it gently until complete dissolution.

Dilute the (10X) concentrate Standard Diluent 10-fold with distilled water to give a 1X working solution. Pour entire contents of the concentrate Standard Diluent into a clean appropriate graduated cylinder. Bring to the final volume with glass-distilled or deionized water. Transfer to a clean wash bottle. Please see example volumes below:

Standard Diluent Concentrate (ml)	Distilled Water (ml)
15	135
25	225

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Preparation of Standard

Standard vials must be reconstituted with the volume of Standard Diluent Buffer 1X shown on the vial immediately prior to use. This reconstitution gives a stock solution of 250 pg/ml of RANTES. Mix the reconstituted standard gently by inversion only. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 250 to 7.81 pg/ml. A fresh standard curve should be produced for each new assay

- Immediately after reconstitution add 200 µl of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 250 pg/ml.
- Add 100 µl of Standard Dilution Buffer to the remaining standard wells B1/ B2 through F1/ F2.
- Transfer 100 µl from wells A1 to B1 and A2 to B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.
- Continue this 1:1 dilution using 100 µl from wells B1 and B2 through to wells F1 and F2 providing a serially diluted standard curve ranging from 250 pg/ml to 7.81 pg/ml.
- Discard 100 µl from the final wells of the standard curve (F1 and F2).

Dilutions can alternatively be performed in clean tubes, and immediately transferred into the relevant wells.

Preparation of Samples

Before testing, human serum must be diluted 1:100 in Standard Diluent Buffer 1X and human plasma samples must be diluted 1:200 in Standard Diluent Buffer 1X.

Preparation of Biotinylated anti-RANTES

It is recommended this reagent is prepared immediately before use. Dilute the biotinylated anti-RANTES with the biotinylated antibody diluent in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes. *Discard diluted detection antibody after use.*

Number of wells required	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a micro centrifuge to collect all the volume at the bottom.

Dilute the 5 µl vial with 0.5 ml of HRP diluent **immediately before use**. *Do not keep this diluted vial for future experiments.* Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial. Please see example volumes. *Discard diluted Streptavidin-HRP conjugate after use.*

Number of wells required	Streptavidin-HRP (µl)	Streptavidin-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

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9. ASSAY METHOD

Prepare all reagents as shown in section 8.

We strongly recommend that every vial is mixed thoroughly without foaming prior to use, except the standard vial which must be mixed gently by inversion only.

Note: Prepare Biotinylated Detection Antibody and Streptavidin-HRP immediately before use.

Assay Step	Details
1. Addition	**Prepare Standard curve** as shown in Section 8 add in duplicate to appropriate wells.
2. Addition	Add 100 µl of each **Sample, zero (Standard Dilution Buffer 1X)** to appropriate number of wells in duplicate.
3. Addition	Add 50 µl of diluted **Biotinylated Anti-RANTES** into all wells.
4. Incubation	Cover with a plastic plate cover and incubate at room temperature (18-25°C) for **1 hour**.
5. Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.3 ml of **1X Wash Solution** into each well. c) Aspirate the contents of each well. d) Repeat step b and c two more times.
6. Addition	Add 100 µl of **Streptavidin-HRP** solution into all wells.
7. Incubation	Cover with a plastic plate cover and incubate at room temperature (18-25°C) for **30 min**.
8. Wash	Repeat wash step 5.
9. Addition	Add 100 µl of ready-to-use **TMB Substrate** into all wells.
10. Incubation	Incubate in the dark for **10-15 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil.
11. Addition	Add 100 µl of **H_2SO_4 Stop Reagent** into all wells.
Read the absorbance value of each well immediately after step 11 on a spectrophotometer using 450 nm as the primary wavelength and 620 nm as the reference wavelength (610 nm to 650 nm are acceptable).	

**Incubation time of the TMB substrate is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore, the color development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.*

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10. DATA ANALYSIS

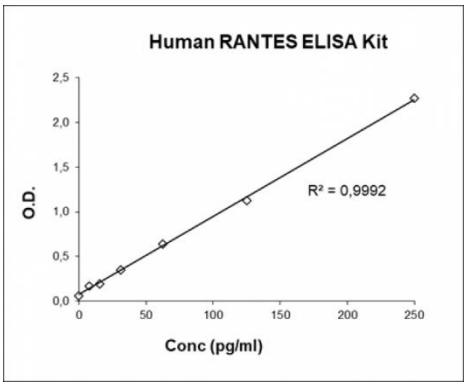
Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding RANTES standard concentration on the horizontal axis.

The amount of RANTES in each sample is determined by extrapolating OD values against RANTES standard concentrations using the standard curve.

Sample RANTES Standard Curve

Std.	RANTES Conc. (pg/ml)	OD (450 nm) Mean	CV (%)
1	250	2.273	1.4
2	125	1.126	1.9
3	62.5	0.637	3.7
4	31.25	0.347	4.1
5	15.63	0.197	0.4
6	7.81	0.166	4.0
Blank	0	0.062	2.7



Note: Curve shown above is an example only and should not be used to determine results.
Every laboratory must produce a standard curve for each set of microwell strips assayed.
For samples human serum or plasmas which have been diluted 1:100 or 1:200 according to the protocol, the calculated concentration should be multiplied by the dilution factor (x100 or x200).

11. ASSAY LIMITATIONS

- Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard Diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples, always multiply results by the appropriate dilution factor to produce actual final concentration.
- The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- As with most biological assays conditions may vary from assay to assay **therefore a fresh standard curve must be prepared and run for every assay.**

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12. PERFORMANCE CHARACTERISTICS

Sensitivity

The minimum detectable dose of RANTES was determined using this Cell Sciences® RANTES ELISA kit was found to be **1.2 pg/ml**. This was determined by adding 2 standard deviations to the mean OD obtained when the zero standard was assayed in 6 independent experiments.

Specificity

The assay recognizes both native and recombinant human RANTES. To define the specificity of this ELISA several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested: IL-2, IL-4R, IL-15, IL-23, IL-34, MCP-1, TRAIL, murine IFNg, murine CCL5 and rat CCL5.

Intra-assay Precision

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates (3 duplicates) of samples containing different concentrations of RANTES:2 in human pooled serum, 2 in culture media and 2 in standard diluent. Data below show the mean RANTES concentration and the coefficient of variation for each sample.

The calculated overall coefficient of variation was 3.4%.

Session	Sample	**Mean** RANTES pg/ml	SD	CV%
Session 1	**Sample 1**	106.58	6.29	**5.9**
	Sample 2	13.14	0.41	**3.2**
	Sample 3	237.98	5.72	**2.4**
	Sample 4	62.81	3.02	**4.8**
	Sample 5	291.75	13.00	**4.5**
	Sample 6	76.25	3.12	**4.1**
Session 2	**Sample 1**	108.11	2.64	**2.4**
	Sample 2	14.42	0.68	**4.7**
	Sample 3	227.23	1.83	**0.8**
	Sample 4	68.38	2.12	**3.1**
	Sample 5	244.23	3.43	**1.4**
	Sample 6	79.90	2.45	**3.1**
Session 3	**Sample 1**	90.41	1.75	**1.9**
	Sample 2	7.55	0.48	**6.3**
	Sample 3	199.45	7.72	**3.9**
	Sample 4	53.19	2.53	**4.8**
	Sample 5	251.34	10.89	**4.3**
	Sample 6	78.19	0.41	**0.5**

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Inter-assay Precision

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments. Each assay was carried out with 6 replicates (3 duplicates) of samples containing different concentrations of RANTES: 2 in human pooled serum, 2 in culture media and 2 in standard diluent. Data below show the mean RANTES concentration and the coefficient of variation for each sample.
The calculated overall coefficient of variation was 7.9%.

	Sample 1	*Sample 2*	*Sample 3*	*Sample 4*	*Sample 5*	*Sample 6*
Mean RANTES pg/ml	101.70	13.78	221.55	61.46	262.44	78.11
SD	9.19	0.86	17.90	7.02	23.82	2.55
CV%	**9.0**	**6.2**	**8.1**	**11.4**	**9.1**	**3.3**

Dilution Parallelism

In two independent experiments, two spiked human serum samples with different levels of RANTES were analyzed at different serial two-fold dilutions (1:2 to 1:16) with two replicates each.

Recoveries ranged from 78 to 140% with an overall **mean recovery of 100%.**

Spike Recovery

The spike recovery was evaluated by spiking two concentrations of RANTES in human serum and culture media in three separate experiments.

Recoveries ranged from 70% to 93% with an overall **mean recovery of 108%.**

Storage Stability

Aliquots of serum samples and spiked medium were stored at –20°C, +2-8°C, room temperature (RT) and at 37°C and the RANTES level determined after 24h. There was no significant loss of RANTES reactivity during storage at +2-8°C, RT and 37°C.

Freeze-thaw Stability

Aliquots of serum samples and spiked medium were stored frozen at –20°C and thawed up to 5 times and the RANTES level was determined. There was no significant loss of RANTES reactivity after 5 cycles of freezing and thawing.

Expected serum values

A panel of 20 human sera and 20 Plasma samples was tested for RANTES. See results below:

Sample Matrix	Number of samples evaluated	Range (ng/ml)	Mean (ng/ml)	Standard Deviation (ng/ml)
Serum	20	[4.61-22.81]	12.11	5.79
Plasma	20	[15.65-37.96]	26.69	6.73

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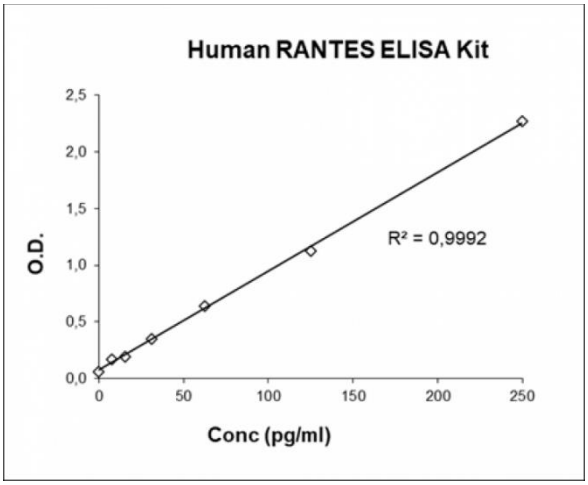

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