ClinMax™ Human CCL5/RANTES ELISA Kit

Catalog Number：CEA-C088

Assay Tests: 96 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing your experiment.

Product information

This kit is specifically designed for the accurate quantitation of human CCL5/RANTES from cell culture supernatants, serum and plasma.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

Manufactured and distributed by
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Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA088-C01	Pre-coated Anti-CCL5 Antibody Microplate	1 plate
CEA088-C02	Human CCL5 Standard	200 µL
CEA088-C03	Biotin-Anti-CCL5 Antibody Con. Solution	100 µL
CEA088-C04	Biotin-Antibody Dilution Buffer	8 mL
CEA088-C05	Streptavidin-HRP Con. Solution	500 µL
CEA088-C06	Streptavidin-HRP Dilution Buffer	15 mL
CEA088-C07	20× Washing Buffer	50 mL
CEA088-C08	2× Sample Dilution Buffer	15 mL×2
CEA088-C09	Substrate Solution	12 mL
CEA088-C10	Stop Solution	6 mL

Storage

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date.
For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

Contents	Storage conditions
Pre-coated Anti-CCL5 Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C.

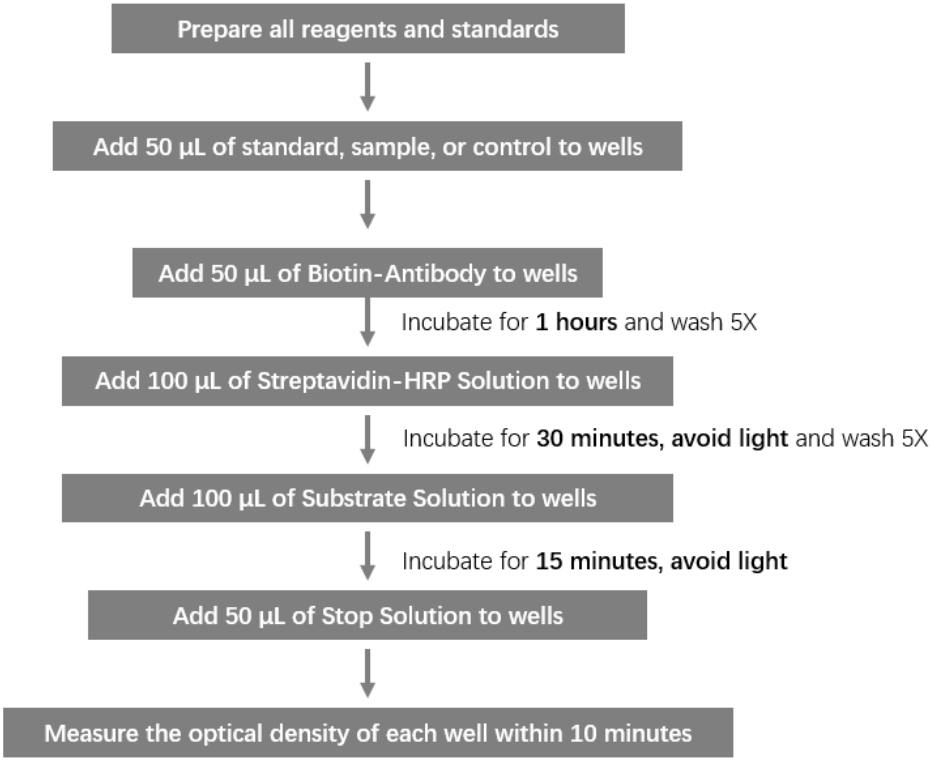
NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

Required materials not supplied.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure or distilled water
Consumables	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

Workflow

Analyte: CCL5/RANTES



NOTE: Incubation temperature is 18 ℃-25 ℃

Prepare the working buffers and standard dilutions.

IMPORTANT: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin-Anti-CCL5 Antibody Solution: Add 60 µL of Biotin-Anti-CCL5 Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. CCL5 Streptavidin-HRP Solution: Add 150 µL of CCL5 Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
4. 1×Sample Dilution Buffer: Dilute 15 mL 2×Sample Dilution Buffer with deionized or ultrapure water to 30 mL.

Prepare the standard serial dilutions.

1. Label 7 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
2. Add 80 µL of the liquid from **Cm** (7500 pg/mL) and 520 µL of Sample Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 =1000 pg/mL).
3. Prepare serial dilutions for the standard curve as follows: Add 300 µL of Sample Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).
4. Transfer 300 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 500 pg/mL).
5. Continue to transfer 300 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7 (15.63 pg/mL).
6. Sample Dilution Buffer serves as zero standard (blank).

Prepare the specimen.

Preparation of sample (Suggested dilution ratio): Labeled a tube “S-1”, add 5 µL of the serum sample and 495 µL of Sample Dilution Buffer to S-1, mix gently well.

PROCEDURE OF ASSAY

1. Add 50 µL of CCL5 Standard, sample, or control to wells.
2. Add 50 µL Biotin-Anti-CCL5 Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hours.**
3. Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
4. Add 100 µL of CCL5 Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for 3**0 minutes, avoid light.**
5. Repeat step 3.
6. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
7. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.

   Note: *the color in the wells should change from blue to yellow.*
8. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes.

   Note: *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

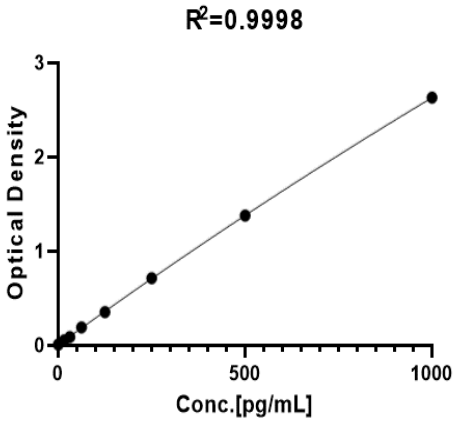
CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample. Then, subtract the average optical density (O.D.) of the zero standard(blank).
2. Establish a standard curve by processing the data using computer software capable of executing a four-parameter logistic (4-PL) curve fitting.
3. Normal range of Standard curve: $R^2 \geqslant 0.9900$.
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

Typical data

Note: *For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.*

CCL5 Standard (pg/mL)	$OD_{450nm-630nm}$
1000	2.633
500	1.380
250	0.717
125	0.357
62.5	0.194
31.25	0.091
15.63	0.056
Blank	0.011



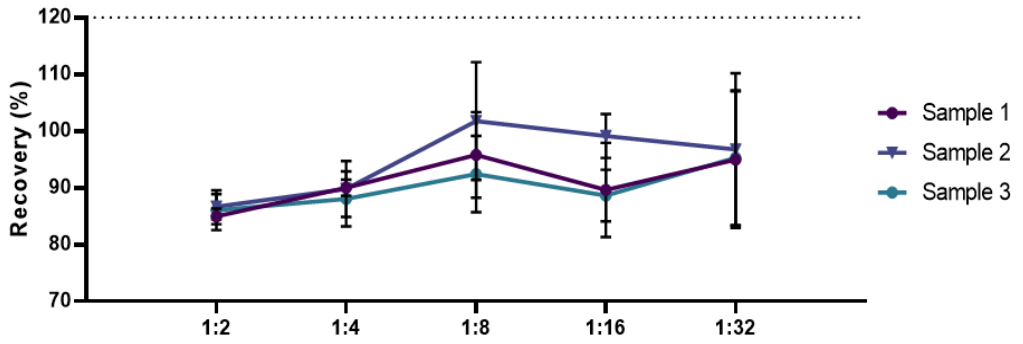
PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of CCL5/RANTES is typically less than 15.63 pg/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

2. Linearity

Three samples (Serum) spiked with high concentrations of CCL5/RANTES were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of CCL5/RANTES for serum samples is 92.0%.



3. Intra-Assay Precision

Ten replicates of each of 4 samples containing different CCL5/RANTES concentrations were tested in one assay. Acceptable criteria: CV < 10%.

Sample Concentration (pg/mL)	Mean (pg /mL)	SD	Numbers	CV
1000	994.9	56.19	10	5.6%
750	698.6	33.99	10	4.9%
500	478.1	22.68	10	4.7%
31.25	26.24	1.360	10	5.2%

4. Inter-Assay Precision

Five samples containing different concentrations of CCL5/RANTES were tested in independent assays. Acceptable criteria: CV<15%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV
1000	994.6	27.07	9	2.7%
500	504.1	10.00	9	2.0%
250	249.9	4.579	9	1.8%
12.5	125.2	4.047	9	3.2%
31.25	34.63	4.292	9	12.4%

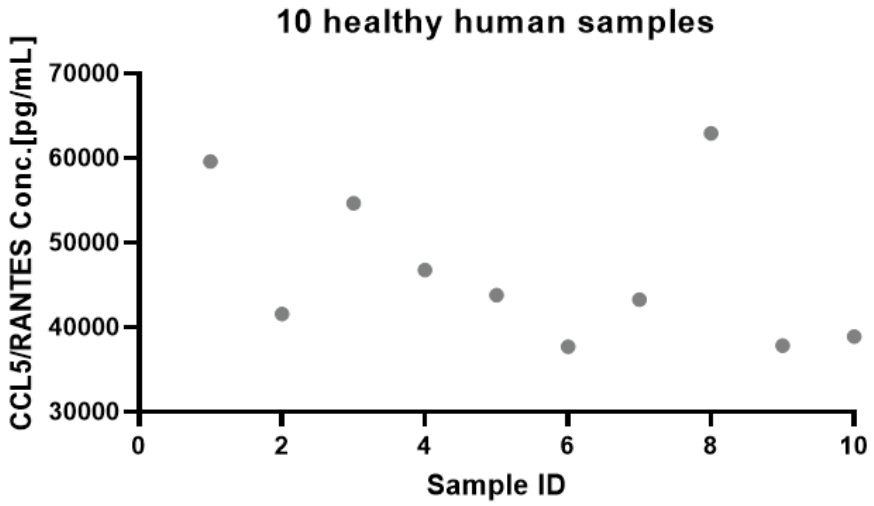
5. Recovery

Recombinant CCL5/RANTES was spiked into 3 human serum samples, and then analyzed. The average recovery of CCL5/RANTES for serum samples is 92.5%.

Sample ID	Conc Measured (pg/mL)	Conc Added(pg/mL)	Conc Recovered (pg/mL)	Recovery
1	990.4	750	864.1	116.9%
	552.3	500	426.0	87.7%
	318.2	250	191.9	81.8%
	126.3	-	-	-
2	954.0	750	760.0	103.9%
	615.5	500	421.6	88.2%
	383.0	250	189.0	83.4%
	194.0	-	-	-
3	949.4	750	772.2	105.3%
	567.4	500	390.2	81.6%
	368.2	250	191.0	83.5%
	177.2	-	-	-

6. Sample Values

10 healthy serum samples were evaluated for the concentrations of human CCL5/RANTES in assay.



TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts