



BioOcean[®] Rat CCL5/RANTES ELISA Kit

Catalog Number: EK3129

Size: 96 Test

For the quantitative determination of rat chemokine (C-C motif) Ligand 5 (CCL5/RANTES) concentrations in cell culture supernates, serum and plasma.

This package insert must be read entirely before using this product. For proper performance, follow the protocol provided with each individual kit.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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QUICK PROCEDURE FLOWCHART

1. Prepare all reagents and standards as directed.



2. Add 300 μ l *Washing Buffer (1 \times)* per well to soak for about 30 seconds. Use immediately after aspirate.



3. Add 100 μ l 2-fold diluted *Standard* to Standard well in duplicate. Add 100 μ l *Assay Buffer (1 \times)* to Blank well in duplicate.



4. Add 100 μ l prediluted sample to the sample well (The dilution refers to the Sample Preparation on Page 6). Step 2 and 3 should be completed within 15 minutes. Incubate for 2 hours at RT.



5. Aspirate and wash 6 times.



6. Add 100 μ l diluted *Detect Antibody* to each well. Incubate for 1 hour at RT.



7. Aspirate and wash 6 times.



8. Add 100 μ l diluted *Streptavidin-HRP* to each well. Incubate for 45 minutes at RT.



9. Aspirate and wash 6 times.



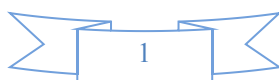
10. Add 100 μ l *Substrate Solution* to each well. Incubate for 10 - 30 minutes at RT. Protect from light.



11. Add 100 μ l *Stop Solution* to each well.



12. Read at 450 nm within 30 minutes. Correction 570 or 630 nm.



DESCRIPTION

Chemokine (C-C motif) ligand 5 (CCL5) also known as RANTES, is a chemotactic cytokine. It is chemotactic for T cells, eosinophils, and basophils, and plays an active role in recruiting leukocytes into inflammatory sites. With the help of particular cytokines (i.e., IL-2 and IFN- γ) that are released by T cells, CCL5 also induces the proliferation and activation of certain natural-killer (NK) cells to form CHAK (CC-Chemokine-activated killer) cells. It is also an HIV-suppressive factor released from CD8⁺ T cells. Recently, the RANTES protein has been engineered for *in vivo* production by Lactobacillus bacteria, and this solution is being developed into a possible HIV entry-inhibiting topical microbicide.

PRINCIPLE OF THE ASSAY

BioOcean Rat CCL5/RANTES ELISA Kit is based on the quantitative sandwich enzyme-linked immunosorbent assay technique to measure concentration of rat CCL5 in the samples. A monoclonal antibody specific for rat CCL5 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and CCL5 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-linked detect antibody specific for CCL5 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, streptavidin-HRP is added. After washing, substrate solution is added to the wells and color develops in proportion to the amount of CCL5 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use expired kit or reagents.
- Do not use reagents from other lots or manufacturers. Do not prepare component by yourself.
- If concentration of assayed factor in samples is higher than the highest standard, dilute the serum/plasma samples with *Assay Buffer*, dilute the cell culture supernate samples with *cell culture medium*. Reanalyze these and multiply results by the appropriate dilution factor.
- Any variation in testing personnel, sample preparation, standard dilution, pipetting technique, washing techniques, incubation time, temperature, kit age and equipment can cause variation in results.
- Bacterial or fungal contamination in either samples or reagents, or cross-contamination between reagents may cause erroneous results.

MATERIALS PROVIDED

Unopened kit should be stored at 2 - 8°C.



- **CCL5 Microplate** (1 plate): 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against rat CCL5.
- **CCL5 Standard** (2 vials): Recombinant rat CCL5 in a buffered protein base with preservatives; lyophilized.
- **CCL5 Detect Antibody** (1 vial, 120 µl): Biotin-conjugate anti- rat CCL5 detect antibody; 100× liquid.
- **Streptavidin-HRP** (1 vial, 150 µl): 100× liquid.
- **Assay Buffer (10×)** (1 bottle, 15 ml): PBS with 0.5 % Tween-20 and 5 % BSA.
- **Substrate** (1 bottle, 15 ml): TMB (tetramethyl-benzidine).
- **Stop Solution** (1 bottle, 15 ml): 0.18 M sulfuric acid.
- **Washing Buffer (20×)** (1 bottle, 50 ml): PBS with 1 % Tween-20.
- **Plate Covers** (5 strips).

STORAGE

Store kit reagents between 2 and 8 °C . Immediately after use remaining reagents should be returned to cold storage (2 to 8°C). Expiry of the kit and reagents is stated on labels. 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, this reagent is not contaminated by the first handling.

Unopened kit		Store at 2 - 8°C (See expiration date on the label).
Opened/ Reconstituted Reagents	1× Washing Buffer 1× Assay Buffer Stop Solution Substrate TMB Detect Antibody Streptavidin-HRP	Up to 1 month at 2 - 8°C.
	Standard	Up to 1 month at ≤ -20 °C in a manual defrost freezer. Discard after use.
	Microplate Wells	Up to 1 month at 2 - 8°C. Return unused strips to the foil pouch containing the desiccant pack, reseal along entire edge to maintain plate integrity.

Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- **Microplate reader** capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- **Pipettes and pipette tips.**
- 50 µl to 300 µl adjustable **multichannel micropipette** with disposable tips.
- Multichannel micropipette **reservoir**.
- **Beakers, flasks, cylinders** necessary for preparation of reagents.
- **Deionized or distilled water.**
- **Polypropylene** test tubes for dilution.

PRECAUTION

- Intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Treat all chemicals with caution because they can be potentially hazardous.
- It is recommended that this product is handled only by persons who have been trained in laboratory techniques and in accordance with the principles of good laboratory practice. Wear personal protection equipment such as laboratory coat, safety glasses and gloves.
- Avoid direct contact with skin or eyes. Wash immediately with water in the case of contact with skin or eyes. Avoid contact of skin or mucous membranes with kit reagents or specimens. See material safety data sheet(s) for specific advice.
- Pure water or deionized water must be used for reagent preparation.
- The Stop Solution provided with this kit is an acid solution. Wear personal protection equipment with caution.
- Do not expose kit reagents to strong light during storage and incubation.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- Use disposable pipette tips and/or pipettes to avoid microbial or cross-contamination of reagents or specimens which may invalidate the test.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the HRP and antibody conjugate.
- Substrate solution must be warmed to room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.
- In some cases, an insoluble precipitate of stabilizing protein has been seen in the Assay Buffer. This precipitate does not interfere in any way with the performance of the test and can thus be ignored. Or remove precipitate by centrifuging at $6,000 \times g$ for 5 minutes.

TECHNICAL HINTS

- All reagents including microplate, samples, standards and working solution should be warmed to room temperature before use.
- To obtain accurate results, using adhesive film to seal the plate during incubation is suggested.
- It is recommended that all samples and standards be assayed in duplicate.
- Avoid foaming when mixing or reconstituting solutions containing protein.
- To avoid cross-contamination, use separate reservoirs for each reagent and change pipette tips between each standard, sample and reagent.
- When using an automated plate washer, adding a 30 seconds soak period before washing step and/or rotating the plate between wash steps may improve assay precision.
- When pipetting reagents, maintain a consistent order of addition from well-to-well.
- Keep Substrate solution protected from direct strong light. Substrate Solution should turn to gradations of blue after a proper color development.
- Read absorbance within 30 minutes after adding stop solution.
- Take care not to scratch the inner surface of the microwells.
- High background maybe occur when using some cell culture medium as standard diluent.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at $\leq -20\text{ }^{\circ}\text{C}$ for later use. Avoid repeated freeze-thaw cycles.

Serum – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at $1,000 \times g$. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at $\leq -20\text{ }^{\circ}\text{C}$ for later use. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at $1,000 \times g$ within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at $\leq -20\text{ }^{\circ}\text{C}$ for later use. Avoid repeated freeze-thaw cycles.

Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay. Dilution with Assay Buffer may be needed.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

If samples are to be run within 24 hours, they may be stored at $2\text{ to }8\text{ }^{\circ}\text{C}$. For longer storage, aliquot samples and store frozen at $-20\text{ }^{\circ}\text{C}$ to avoid loss of bioactive rat CCL5. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Normal serum and plasma samples require a 200-fold dilution. A suggested 200-fold dilution is 5µl sample + 995 µl Assay Buffer (1×).

REAGENT PREPARATION

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with distilled water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25°C. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (15 ml) of the **Assay Buffer (10×)** into a clean 250 ml graduated cylinder. Bring to final volume of 150 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8°C. Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.

Make a **1: 100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Detect Antibody should be used within 30 minutes after dilution.

Streptavidin-HRP

Mix well prior to making dilutions.

Make a **1: 100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Streptavidin-HRP should be used within 30 minutes after dilution.

Sample Dilution

If your samples have high CCL5 content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

Rat CCL5 Standard

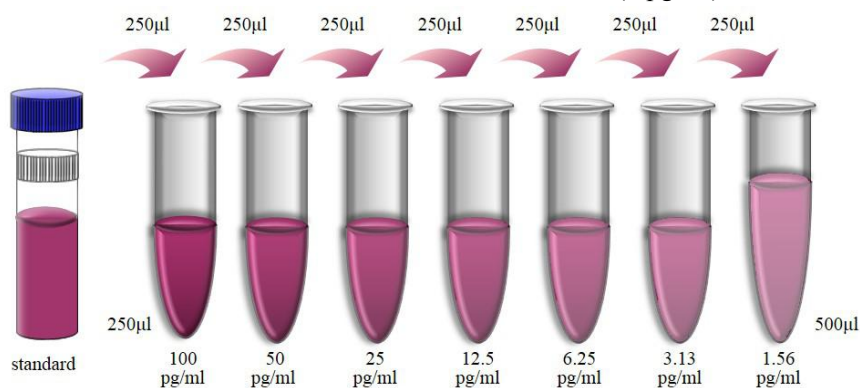
Reconstitute **Rat CCL5 Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 200 pg/ml).

Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

Use polypropylene tubes.

For serum/plasma samples, mixing *concentrated rat CCL5 standard* (250 µl) with 250 µl of *Assay Buffer (1×)* creates the high standard (100 pg/ml). Pipette 250 µl of *Assay Buffer (1×)* into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. *Assay Buffer (1×)* serves as the zero standard (0 pg/ml).

For cell culture supernates, mixing *concentrated rat CCL5 standard* (250 µl) with 250 µl of cell culture medium creates the high standard (100 pg/ml). Pipette 250 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.
2. Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.
3. Add 300 µl *Washing Buffer (1×)* per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess *Washing Buffer (1×)*. Use the microwell strips immediately after washing. **Do not allow wells to dry.**
4. Add 100 µl of 2-fold diluted *Standard* to Standard well in duplicate. Add 100 µl of *Assay Buffer (1×)* to Blank well in duplicate.
5. Add 100 µl prediluted sample to the sample well (The dilution refers to the Sample Preparation on Page 6). Ensure reagent addition is uninterrupted and completed within 15 minutes.
6. Seal the plate with a fresh *adhesive film*. Incubate at room temperature (18 to 25°C) for 2 hours on a microplate shaker set at 300 rpm.
7. Aspirate each well and wash by filling each well with 300 µl *Washing Buffer (1×)*, repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining *Washing Buffer (1×)* by aspirating or decanting. Invert the plate and tap it against clean paper towels.
8. Add 100 µl of diluted *Detect Antibody* to each well.
9. Seal the plate with a fresh *adhesive film*. Incubate at room temperature (18 to 25°C) for 1 hour on a microplate shaker set at 300 rpm.
10. Repeat aspiration/wash as in step 7.
11. Add 100 µl of diluted *Streptavidin-HRP* to each well.

12. Seal the plate with a fresh *adhesive film*. Incubate at room temperature (18 to 25 °C) for 45 minutes on a microplate shaker set at 300 rpm.
13. Repeat aspiration/wash as in step 7.
14. Add 100 µl of *Substrate Solution* to each well. Incubate for 10 - 30 minutes at room temperature. Protect from light.
15. Add 100 µl of *Stop Solution* to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
16. Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

CALCULATION OF RESULTS

Average the duplicate optical density readings for each standards and sample, then subtract the average optical density value of the zero standard.

Standard Concentration as horizontal axis, optical density (OD) Value as the vertical axis, regressing the data and create a standard curve using computer software. The data may be linearized by plotting the log of the CCL5 concentrations versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

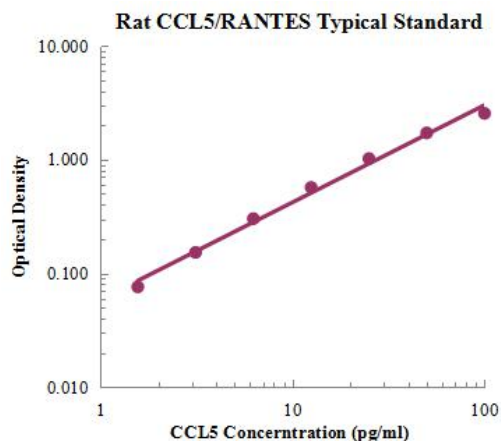
Note: The finally concentration of top standard is 100 pg/ml. If instruction in this protocol have been followed samples have been diluted by 1:200 ratio (5µl sample + 995 µl Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (×200).

If serum/plasma samples have been diluted following the instruction, the final dilution factor is 200.

TYPICAL DATA

A standard curve must be run within each assay. This standard curve is provided for demonstration only.

pg/ml	O.D.	Average	Corrected	
0.00	0.035	0.029	0.032	
1.56	0.109	0.107	0.108	0.076
3.13	0.184	0.185	0.185	0.153
6.25	0.327	0.341	0.334	0.302
12.50	0.601	0.599	0.600	0.568
25.00	1.036	1.063	1.050	1.018
50.00	1.729	1.757	1.743	1.711
100.00	2.582	2.560	2.571	2.539



SENSITIVITY

The minimum detectable dose (MDD) of CCL5 is typically about 0.36 pg/ml. The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

Intra-assay Precision (Precision within an assay)

Three serum-based and buffer-based samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three serum-based and buffer-based samples of known concentration were tested in six separate assays to assess inter-assay precision.

	Intra-assay precision			Inter-assay precision		
Sample	1	2	3	1	2	3
n	20	20	20	6	6	6
Mean (pg/ml)	3.3	12.8	51.0	3.3	13.6	53.7
Standard deviation	0.1	0.3	1.8	0.2	0.7	2.4
CV (%)	3.9	2.4	3.5	5.2	4.9	4.5

RECOVERY

The spike recovery was evaluated by spiking 3 levels of rat CCL5 into five health rats serum samples. The un-spiked serum was used as blank in these experiments. The recovery ranged from 88 % to 122 % with an overall mean recovery of 103 %.

LINEARITY

To assess the linearity of the assay, five serum samples were spiked with high concentration of CCL5 in rats serum and diluted with Assay Buffer to produce samples with values within the dynamic range of the assay.

	Average (%)	Range (%)
1:2	102	92 - 112
1:4	105	95 - 118
1:8	104	94 - 114
1:16	108	100 - 117

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy rats were evaluated for the presence of CCL5 in this assay.

Sample Matrix	Number of Samples Evaluated	Range (ng/ml)	Detectable (%)	Mean of Detectable (ng/ml)
Serum	30	1.2 - 6.3	100	2.6

Note: The sample range is non-physiological range. The sample range of healthy rats will difference according to geographical, ethnic, sample preparation, and testing personnel, equipment varies. The above information is only reference.

SPECIFICITY

This kit could assay both natural and recombinant rat CCL5. A panel of substances listed below were prepared at 1 ng/ml in Assay Buffer to determine cross-reactivity. Preparations of the following substances at 1 ng/ml in a mid-range rrCCL5 control to determine interference. No significant cross-reactivity or interference was observed.

Human	Mouse	Rat
MEC	C-10	MCP-1
RANTES	Eotaxin	MIP-1 α
TECK	JE	MIP-1 β
	MCP-2	
	MCP-3	
	MCP-5	
	MEC	
	MIP-1 α	
	MIP-1 β	
	MIP-1 γ	
	MIP-2	
	MIP-3 α	

PLATE LAYOUT

