Mouse CCL5/RANTES ELISA Kit Instruction Manual

Specification: 96T/48T
Cat#: FK-1300

Purpose: Used to detect the concentration of mouse Chemokine (C-C motif) ligand 5 (CCL5/RANTES) in serum, plasma, cell culture supernatant, and other samples.

Please read the instructions carefully before use. If you have any questions, please contact us through the following ways:

E-Mail: marketing@fantibody.com

Company website: https://shops.fantibody.com

Please refer to the label on the outer packaging of the kit for the specific expiration date. Use the kit within the expiration date. When contacting us, please provide the product number and production date (see box label) to help us serve you more efficiently.

Kit Performance

Physical Performance: All liquid components should be clear and transparent without any precipitation or flocculent matter. The microplate aluminum foil bag should be vacuum-packed without damage or air leakage.
Calibration Curve Linearity: The correlation coefficient (r) of the calibration curve should be greater than or equal to 0.9900.
Precision: Intra-assay coefficient of variation (CV%) should be less than 10%; inter-assay CV% should be less than 15%.
Sensitivity: The minimum detectable dose should be less than 39.062 pg/mL.
Recovery Rate: Recovery rate should be between 85%-115%.
Specificity: This kit recognizes natural mouse CCL5 and does not cross-react with similar structures.
Stability: Store at 2℃-8℃, valid for 6 months.
Detection Range: 156.25 pg/mL-5000 pg/mL.

Principle of the Assay

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). The microplate is pre-coated with an anti- mouse CCL5 antibody (solid-phase antibody). Mouse CCL5 standards and samples are added to the wells, followed by a biotin-labeled antibody. After incubation and thorough washing, HRP-conjugated streptavidin is added. After further incubation and washing, unbound components are removed, forming a sandwich complex of solid-phase antibody-antigen-biotin-labeled antibody-streptavidin-HRP on the microplate. TMB substrate is added, producing a blue product that turns yellow under the action of the stop solution. The absorbance (OD value) is measured at 450nm using a microplate reader. The OD value is positively correlated with the concentration of mouse CCL5 in the sample. By fitting the

standard curve, the concentration of mouse CCL5 in the sample can be calculated.

Kit Components and Storage

Component	Quantity	Main Ingredients
High Standard	2 vials	Lyophilized powder
Reconstitution Solution	2 vials	PBS
Standard & Sample Diluent	25mL	PBSTN
Microelisa Strip plate	96T/48T	Pre-coated Solid Phase Antibody
Bio-Antibody	10mL	Biotin Antibody
HRP-Conjugate Reagent	10mL	HRP-Conjugated Avidin
TMB	10mL	TMB substrate
Stop Solution	6mL	Acidic Solution
20×Wash Solution	25mL	0.05%Tween20
Instructions	1copy	--
Ziplock Bag	1piece	--
Sticker	4pieces	--

Note:

1. Before use, check that the labels and quantities of the reagents in the kit match the table.
2. Store the kit at 2-8℃. Do not use expired kits.
3. If the microplate is not used completely in one experiment, seal and store it at 2-8℃.
4. Reconstituted standards should be used on the same day.
5. If the kit components need to be reused, ensure they are not contaminated after the last use.

Required but Not Provided Equipment (can assist with purchase)

1. Standard microplate reader.
2. Automatic plate washer.
3. Shaker.
4. Series of adjustable pipettes and tips. For large sample sizes, a multi-channel pipette is recommended.

Kit Limitations

1. For research use only, not for clinical diagnosis.
2. Use within the indicated expiration date, do not use expired products.
3. Do not mix components from different kits or manufacturers.
4. Use the sample diluent provided with the kit.
5. If the sample value exceeds the highest standard concentration, dilute the sample appropriately and re-test.
6. Interference from human anti-mouse antibodies in the sample can affect results. Exclude this factor before testing.
7. Results obtained by other methods are not directly comparable to those obtained with this kit.

Precautions

1. This kit is for in vitro research use only, not for clinical diagnosis.
2. Wear lab coats and latex gloves for protection during the experiment, especially when handling blood or other bodily fluids. Follow national biosafety guidelines.
3. Incubate at the specified time and temperature to ensure accurate results. All reagents must reach room temperature (20-25℃) before use. Store reagents immediately after use.
4. Incorrect washing can lead to inaccurate results. Ensure to dry the wells thoroughly before adding the substrate. Do not let the wells dry out during incubation.
5. Remove any residual liquid and fingerprints from the bottom of the plate to avoid affecting OD values.
6. The substrate should be colorless or very light in color.
7. Avoid cross-contamination of reagents and samples to prevent erroneous results.
8. Avoid direct exposure to strong light during storage and incubation.
9. Allow the plate to equilibrate to room temperature before opening the sealed bag to prevent condensation.
10. Do not allow any reaction reagents to come into contact with bleach or strong bleach fumes, as these can destroy the biological activity of the reagents.
11. The microplate reader should be equipped with a filter capable of detecting at 450 ± 10nm wavelength, with an optical density range of 0-3.5. Preheat the microplate reader for 15 minutes before use.
12. Do not mix or substitute reagents from different batches or sources.
13. Use disposable EP tubes and tips for each experiment and do not reuse them.
14. Do not use expired reagents.

Sample Preparation and Storage

The following is a general guideline for sample collection and storage. Do not use sodium azide as a preservative. If samples are not analyzed immediately, aliquot and freeze them to avoid repeated freeze-thaw cycles.

Cell Culture Supernatant: Centrifuge to remove precipitates, analyze immediately or aliquot and freeze at -20℃.
Serum: Collect blood in a clean tube, allow to clot at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect the serum. Analyze immediately or aliquot and freeze at -20℃.
Plasma: Use heparin, citrate, or EDTA as anticoagulants. Centrifuge at 2000×g for 20 minutes within 30 minutes of collection at 2-8 ℃. To eliminate platelet effects, further centrifuge at 10000×g for 10 minutes at 2-8℃. Analyze immediately or aliquot and freeze at -20℃.
Cell Lysate: For adherent cells, remove the culture medium, wash once with PBS, saline, or serum-free medium. Add an appropriate amount of lysis buffer, pipette up and down to ensure full contact. Cells usually lyse within 10 seconds. For suspension cells, collect by centrifugation, wash once with PBS, saline, or serum-free medium. Add an appropriate amount of lysis buffer, pipette to disperse cells, flick with fingers to ensure full lysis. After full lysis, centrifuge at

10000-14000 × g for 3-5 minutes, and collect the supernatant. Analyze immediately or aliquot and freeze at -20℃.

Urine: Collect in a sterile tube, centrifuge at 2000 × g for 20 minutes. Carefully collect the supernatant. If precipitates form, centrifuge again.

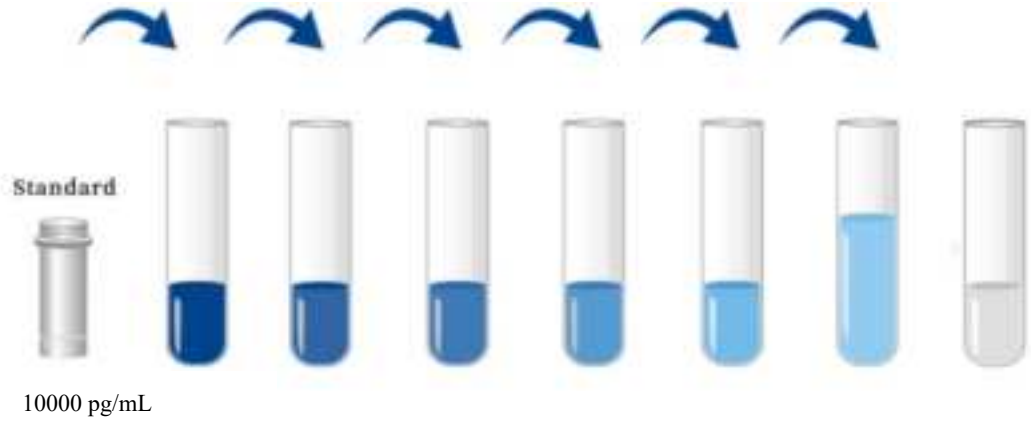
Reagent Preparation

1. Before use, allow all components to equilibrate to room temperature for at least 120 minutes.
2. Concentrated Wash Solution: Crystals may form in the concentrated wash solution when stored in the refrigerator, which is normal. Dissolve the crystals completely by warming in a water bath. Dilute the concentrated wash solution with distilled water at a ratio of 1:20, i.e., add 19 parts of distilled water to 1 part of concentrated wash solution.

Standard Dilution Method

Reconstitution of Standards: Reconstitute the standard by adding the entire volume of the reconstitution solution to the lyophilized standard vial. Gently vortex to ensure thorough mixing. The concentration of the reconstituted stock solution is 10000 pg/mL. Mix thoroughly before dilution.

Dilution of Stock Solution: Allow the reconstituted standard to stand for 1-2 minutes. Dilute the stock solution with the standard & sample diluent in a series of dilutions. Take 7 EP tubes, each containing 500 μL of standard & sample diluent. Add 500 μL of the 10000 pg/mL stock solution to the first tube to make a 10000 pg/mL working solution. Continue this stepwise dilution to achieve the desired concentrations as shown in the diagram below.



Suggested Standard Concentrations: Prepare the standards at the following concentrations: 5000, 2500, 1250, 625, 312.5, 156.25, 0 pg/mL. Use these concentrations to plot the standard curve.

Note: The last tube should contain only the standard & sample diluent as the 0 pg/mL standard. Prepare the working solutions fresh before use.

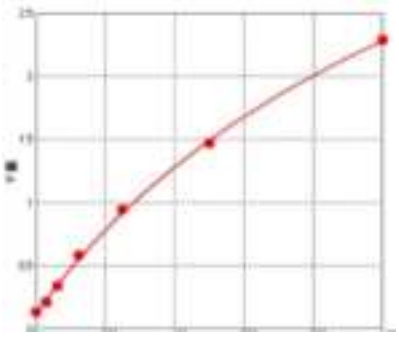
Assay Procedure

Recommended Sample Dilution: Perform a preliminary experiment to determine the optimal sample dilution before conducting the formal experiment.

1. Allow all reagents and components to reach room temperature. Prepare working solutions of all components as described in the manual.
2. Remove the required strips from the aluminum foil bag. Seal the remaining strips in a ziplock bag and store at 2-8℃.
3. Set up wells for standards, sample diluent, blank, and samples. Add 50 μL of each standard concentration to the respective wells. Add 50μL of sample diluent to the sample diluent wells. Leave the blank wells empty. Add 50μL of each sample to the sample wells. Add 100 μL of biotin-labeled antibody to all wells except the blank wells. Cover the plate with a plate sealer and incubate at 37℃for 60 minutes.
4. Remove the plate sealer, discard the liquid, and pat dry on absorbent paper. Fill each well with wash solution, let stand for 1 minute, discard the wash solution, and pat dry. Repeat this washing step 5 times. If using an automatic plate washer, follow the operating instructions and include a 30-second soak step to improve accuracy. After washing, pat the plate dry on clean, lint-free paper.
5. Add 100 μL of HRP-conjugated streptavidin to each well except the blank wells. Cover the plate with a plate sealer and incubate at 37℃ for 20 minutes.
6. Repeat the washing step as described in step 4.
7. Add 100 μL of TMB substrate to each well. Cover the plate with a plate sealer and incubate at 37℃ for 15 minutes.
8. Add 50 μL of stop solution to each well. Read the absorbance at 450 nm using a microplate reader.

Result Calculation

1. Plot the standard curve using the standard concentrations as the x-axis and the corresponding absorbance (OD value) as the y-axis. Use computer software to fit the curve using a four-parameter logistic (4-PL) model. Calculate the sample concentrations based on the standard curve equation.
2. If the samples were diluted, multiply the calculated concentration by the dilution factor to obtain the final sample concentration.



(**The diagram is for reference only**)

Operation Summary

Biotin

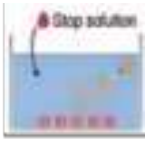
1、Add 50 μL of standard working solution or sample to the wells of the reaction plate, then immediately add 100μL of biotin-labeled antibody working solution to each well. incubate at 37℃ for 60 minutes.

2、Discard the liquid from, the plate and wash the plate 5 times.

3、Add 100 mL of HRP-conjugated working solution to each well and incubate at 37c for 20 minutes Discard the liquid from the plate and wash the plate 5 times.

streptavdin

4、Add 100μL of TMB substrate to each well and incubate at 37℃ for 15 minutes.



5、Add 50 μL of stop solution to each well.

6、 Immediately read the absorbance 450 nm and process the data.

Troubleshooting

Problem	Possible Cause	Solution
Unstable Positive/negative control results	Inaccurate pipetting	Check pipettes and tips
	Insufficient equilibration time	Ensure sufficient equilibration time
	Incomplete washing	Ensure proper washing time and volume per well
Weak or no color development	Insufficient incubation time	Ensure sufficient incubation time
	Incorrect experimental temperature	Use the recommended temperature
	Insufficient reagent volume or omission	Check pipetting and addition process
	Incorrect dilution	Ensure correct dilution

	Inactivated enzyme or substrate	Mix enzyme conjugate and substrate, check for rapid color development
Low OD values	Incorrect microplate reader settings	Check wavelength and filter settings, preheat reader
		Turn on the microplate reader in advance to warm up
High variation coefficient	Inaccurate pipetting	Check pipetting accuracy
High background	Excessive antibody concentration	Use recommended dilution
	Incomplete washing	Ensure complete washing; check for blockages in automatic washer; use kit wash solution
	Contaminated wash solution	Prepare fresh wash solution
Low sensitivity	Improper storage of ELISA kit	Store reagents as per instructions
	No stop solution added before reading	Add stop solution before reading OD

Declaration

1. Due to current conditions and scientific limitations, not all raw materials can be fully identified and analyzed. This product may have certain quality and technical risks.
2. This kit has been developed to remove/reduce some endogenous interfering factors in biological samples, but not all possible factors have been removed.
3. The final experimental results are closely related to the validity of the reagents, the operator's technique, and the experimental environment. The company is only responsible for the kit itself and not for sample consumption due to kit use. Users should consider the potential sample usage and reserve sufficient samples.
4. To achieve the best experimental results, use only the reagents provided in the kit and do not mix with products from other manufacturers. Follow the instructions strictly.
5. Incorrect reagent preparation or microplate reader settings may lead to abnormal results. Read the instructions carefully and adjust the equipment before the experiment.
6. Even with the same operator, different results may be obtained in two independent experiments. To ensure reproducibility, control each step of the experiment.
7. The kit undergoes strict quality control before shipment. However, due to transportation conditions, differences in experimental equipment, etc., user results may differ from factory data.
8. This kit has not been compared with similar kits from other manufacturers or different methods for detecting the same target, so inconsistent results cannot be ruled out.
9. This kit is for research use only. If used for clinical diagnosis or any other purpose, the company is not responsible for any resulting issues and does not assume any legal liability.