



(For scientific research use only, not for clinical diagnosis!)

**Rat Nuclear Factor κ B Receptor Activator Ligand Antibody
(RANKL-Ab) ELISA Kit Instructions for Use Product No.: BY-
ER339079 Specifications: 48T/96T**

**Please read the instructions carefully before use. If you have any questions,
please contact us through the following methods: Official hotline: 025-5229-
8998 Sales department phone: 13914481711 Technical phone: 15950492658
Contact email: 3224949330@qq.com Company website:
www.byabscience.cn For specific shelf life, please see the reagents Box
packaging label. Please use the kit within the shelf life.**

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

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Kit performance Detection range: 3.75

ng/mL– 120 ng/mL.

Sensitivity: The lowest detectable dose is less than 0.1 ng/mL.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

Recovery rate: The recovery rate is between 85%-115%.

Specificity: This kit recognizes natural and recombinant rat receptor activator of nuclear factor kappa B ligand antibody (RANKL-Ab) and has no crossover with structural analogs.

Stability: Stored at 2°C-8°C, validity period is 6 months.

Purpose: Used to detect the concentration of rat nuclear factor kappa B receptor activating factor ligand antibody (RANKL-Ab) in samples such as serum, plasma, cell culture supernatant and tissue.

Shelf life: Stored at 2°C-8°C, valid for 6 months.

Experimental principle

The kit uses an indirect enzyme-linked immunosorbent assay (ELISA). To the coated microwells pre-coated with rat nuclear factor κ B receptor activator ligand antibody (RANKL-Ab) capture antigen, add the sample to be tested and the standard in sequence, then add the HRP-labeled detection antibody, and incubate. and wash thoroughly. Adding substrates A and B, the substrates are catalyzed by HRP to produce a blue product, which is finally converted into yellow under the action of the stop solution (acidic solution). The absorbance (OD value) was measured at a wavelength of 450 nm using a microplate reader. The absorbance (OD value) was positively correlated with the concentration of rat

nuclear factor κ B receptor activator ligand antibody (RANKL-Ab) in the sample to be tested. By fitting the calibrator curve, the concentration of rat nuclear factor κ B receptor activator ligand antibody (RANKL-Ab) in the sample can be calculated.

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Kit components and storage: Store unopened kits at 2-8 degrees Celsius. Do not use expired kits.

Components	48-well configuration	96-well configuration	Store after opening
Pre-coated enzyme plate	48T	96T	2-8°C 14 days
Standard product	0.3mL*6 tubes	0.3mL*6 tubes	2-8°C 14 days
sample diluent	3ml	6ml	2-8°C 180 days
HRP labeled antibodies	5ml	10ml	2-8°C 14 days
Chromogenic substrate A	3ml	6ml	2-8°C 180 days
Chromogenic substrate B	3ml	6ml	2-8°C 180 days
stop solution	3ml	6ml	2-8°C 180 days
20×Lotion	15ml	25ml	2-8°C 180 days
sealing film	2 sheets	2 sheets	
manual	1 serving	1 serving	
Ziplock bag	1	1	

The concentrations of calibrators are: 120, 60, 30, 15, 7.5, 3.75 ng/mL.

Note: 1: Before use, please check whether the label and quantity of

the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have

not been contaminated since the last use. 3: If the enzyme plate is not used up in a

single time, remember to seal it and store it at 2-8°C.

Prepare your own test equipment required for the test (not provided, but can assist in

1) Microplate reader capable of detecting

absorbance at 450 nm 2) Pipette, pipette tip,

and sample addition tank 3) 37°C incubator

or water bath 4) Test tubes, centrifuge tubes,

measuring cylinders, etc. for preparing

reagents 5) Distilled water or deionized

water Ionized water

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6) Vortex shaker, microplate shaker

**Notes 1) For scientific research use only,
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- 2) Use within the validity period marked on the kit. Expired products must not be used.
- 3) Do not mix with kits or components from other manufacturers. Use the sample diluent provided with the kit.
- 4) If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure.
- 5) Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.
- 6) The test results obtained by other methods are not directly comparable to the test results of this kit.
- 7) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.
- 8) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.
- 9) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.
- 10) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.
- 11) The substrate chromogenic solution should be colorless or very light in color.
- 12) Avoid cross-contamination of reagents and specimens to avoid erroneous results.

13) Avoid direct exposure to strong light during storage and incubation.

14) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of $450\pm 10\text{nm}$, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.

15) The EP tubes and suction tips used in the test are single-use and are strictly prohibited from mixing.

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Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them once with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

Tissue homogenate - rinse the tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate will affect the measurement results), weigh and cut the tissue into pieces. Mix the minced tissue with the corresponding volume of PBS (generally

according to the weight to volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS. The specific volume can be adjusted appropriately according to the experimental needs, and recorded. It is recommended to add Protease inhibitor) was added to a glass homogenizer and ground thoroughly on ice. In order to further lyse tissue cells, the homogenate can be sonicated or frozen and thawed repeatedly. Finally, centrifuge the homogenate at 5000 × g for 5 to 10 minutes, and take the supernatant for detection.

Urine - Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

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Reagent preparation 1. Before use, all components must be rewarmed for at least 60 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water.

3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

Operating procedures: Return all reagents and components to room temperature first. For standards, quality control materials and samples, it is recommended to make duplicate holes. 1. Prepare the working solutions of various components of the kit according to the method described in the previous instructions.

2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

3. Set up standard wells and sample wells, and add 50 μL of standards of different concentrations to each standard well;

4. Add 50 μL of the sample to be tested into the sample well; do not add it to the blank well.

5. Except for the blank well, add 100 μL of horseradish peroxidase (HRP)-labeled detection antibody to each well of the standard well and sample well, seal the reaction well with a sealing film, and keep the temperature in a water bath or constant temperature oven at 37°C. Incubate for 60 minutes.

6. Discard the liquid, pat dry on absorbent paper, fill each well with washing solution (350 μ L), let it stand for 1 minute, shake off the washing solution, pat dry on absorbent paper, and repeat washing the plate 5 times (you can also use a plate washer to wash it) plate).

(Tip: In order to obtain ideal experimental results, the residual liquid must be completely removed. After washing the plate, please proceed to the next step immediately and do not let the microplate dry.) 7. Add 50 μ L each of substrates A and B to each well. Incubate at 37°C in the dark for 15 minutes.

8. Add 50 μ L of stop solution to each well, and within 15 minutes, measure the OD value of each well at a wavelength of 450 nm.

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[Result calculation]

9. Use the concentration of the standard substance as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software]
10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample. Note: Experimenters need to establish a standard curve based on their own experiments. For each test, a standard curve must be established for each enzyme plate. The following curves are for reference only!



(Schematic diagram of the music, for reference only)

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[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used strips and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information: [Questions and Answers]

Problem description	Possible reasons	Corresponding countermeasures Corresponding countermeasures
standard curve gradient difference	Incorrect liquid aspiration or	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washes and the amount of liquid added to each hole
Very weak or colorless	Incubation time too short	Ensure adequate incubation time
	Experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspirating and adding process to ensure that all reagents are added in order and in
	Incorrect dilution	
	Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid color development
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the	Use the recommended dilution
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked;
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to

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