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Human natural killer cells (NK) ELISA Kit

Product Number: ELK059

Shipping and Storage

1. Reagent kit storage: 2-8℃.
2. Validity period: 6 months.

Component

Component	48T
Microplate	8 wells×6 strips
Calibration standard (10 ×)	100μL
Enzyme marker (100 ×)	50μL
Universal diluent (20 ×)	15mL
Concentrated washing solution (20 ×)	25mL
Chromogen substrate	12mL
Stop solution	6mL

If you need to purchase universal components separately, please provide the corresponding component names.

Description

The reagent kit adopts the principle of double antibody sandwich method: the capture antibody is wrapped on the enzyme-linked immunosorbent assay (ELISA) plate, and the analyte in the sample and calibration sample is captured. The enzyme-linked secondary antibody labeled with horseradish peroxidase is added to form an "antibody antigen antibody HRP" immune complex. After adding TMB for color development, if the analyte in the sample appears blue, the reaction is stopped by adding termination solution. During the detection process, free components are washed away, and the OD value is measured at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader. The color intensity is proportional to the content of the analyte in the sample, and the concentration of the analyte in the sample is calculated by plotting a standard curve.

Application

This kit is used to detect the concentration of human natural killer (NK) antigen in serum, plasma and other samples for research purposes only.

Testing Scope

0.312-20ng/mL

Required but not included experimental materials

1. Distilled water or deionized water, disposable centrifuge tubes, disposable gloves and other consumables;
2. Pipettes, multi-channel pipettes, and matching gun heads;
3. Beakers, measuring cylinders, reagent bottles and other containers;
4. Sealing film or other alternative materials used for sealing microporous plates;
5. Microplate oscillator (if needed), centrifuge, vortex oscillator and other auxiliary equipment;
6. Constant temperature incubator, constant temperature water bath, enzyme-linked immunosorbent assay reader, washing machine.

Preparation and storage of reagents

1. Preparation of 1×Wash Solution: If crystals precipitate from the concentrated wash solution (20×), it needs to be heated at 37℃

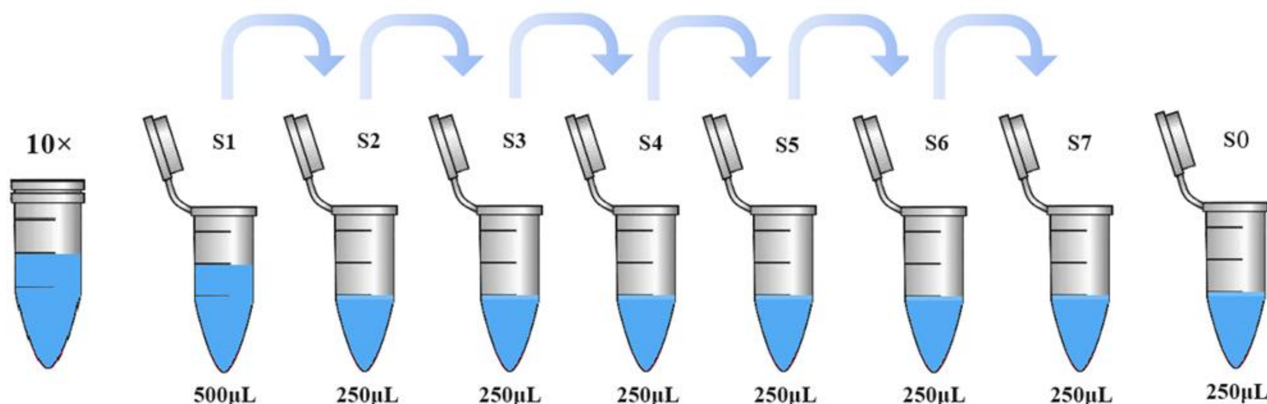
until all crystals are dissolved before use. Dilute with distilled water at a ratio of 1:20, for example: add 10mL of concentrated washing solution to 190mL of distilled water and mix evenly.

Preparation of 1×universal diluent: Dilute with distilled water at a ratio of 1:20. For example, add 10mL of 20×concentrated universal diluent to 190mL of distilled water and mix evenly.

Preparation of 1×enzyme labeled working solution: Dilute 100 × enzyme labeled antibody with "1 × universal diluent" at a ratio of 1:100. For example, add 990uL of "1 × universal diluent" to 10uL of (100 ×) enzyme labeled antibody and mix evenly. The prepared 1×enzyme labeled antibody working solution can be stored at 2-8℃ for 8 hours.

2. Preparation of calibration samples for work: Before opening, the calibration samples should be centrifuged for 30 seconds to ensure that all calibration samples are concentrated at the bottom;

   Prepare 7 centrifuge tubes and draw a certain amount of 10×calibration standard as needed. Dilute it 10 times with "1× universal diluent" to prepare calibration standard S1. For example, prepare 500μL of S1 concentration calibration standard by adding 50uL of 10×calibration standard and 450uL of "1 x universal diluent". Add 250μL of "1× universal dilution solution" to the following 6 centrifuge tubes, and dilute the S1 calibration sample in these 6 tubes (S2~S7) by multiple ratios for 6 gradients to S7. A total of 7 concentrations of calibration samples were prepared, namely S1, S2, S3, S4, S5, S6, and S7, as shown in the figure below. "1 × universal dilution solution" was used as the blank calibration sample S0.



Sample collection, preprocessing, and storage

1. Serum: Collect whole blood using a serum collection tube, let it stand at room temperature for blood cell agglutination, or centrifuge directly at 1000×g for 15 minutes to obtain serum. The operation should be gentle to avoid hemolysis. The obtained serum should be tested in a timely manner. If it cannot be tested in a timely manner, it should be divided into multiple parts and stored at a temperature of -20℃ or below for no more than 6 months. The sample should be frozen and thawed no more than twice.
2. Plasma: Collect plasma using EDTA or heparin blood collection tubes. After collecting whole blood, centrifuge at 1000×g for 15 minutes to obtain plasma. The operation should be gentle to avoid hemolysis. The obtained plasma should be tested in a timely manner. If it cannot be tested in a timely manner, it should be divided into multiple parts and stored at a temperature of -20℃ or below for no more than 6 months. The sample should be frozen and thawed no more than twice.
3. Organization: Weigh the tissue and cut it into pieces. Add the cut tissue to the lysis buffer, usually in a weight to volume ratio of 1:4-1:9. For example, 100mg of tissue sample corresponds to 400uL of lysis buffer, which can be adjusted appropriately according to experimental needs. Finally, centrifuge the homogenate at 5000×g for 5-10 minutes and take the supernatant for detection.
4. Cells: Take an appropriate amount of cells, sonicate on ice, centrifuge at 5000×g for 5-10 minutes, and take the supernatant for detection.
5. Cell supernatant: Take the cell supernatant and centrifuge at 5000×g for 5-10 minutes. Take the supernatant for detection.

Protocol

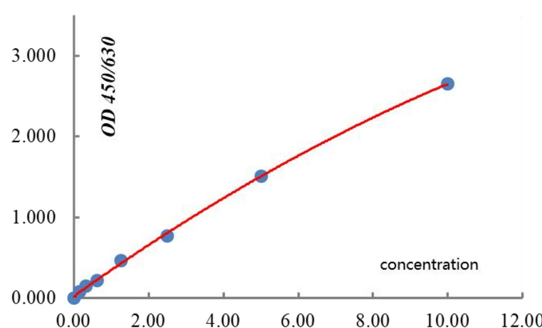
Before use, allow all reagents to equilibrate at room temperature for about 30 minutes, or quickly return to room temperature in a 37℃ incubator.

Note: Do not open the enzyme-linked immunosorbent assay (ELISA) plate until it has returned to room temperature.

1. Number: Calibration wells, sample wells, and blank wells need to be set up for testing, and the arrangement of each sample should be reasonably planned.
2. Dilution: Calibration well: Add 100µL of calibration standard to each well (S1-S7, a total of 7 concentrations).
3. Sample addition: Sample well: Add 50µL of 1×universal diluent to each well, followed by 50uL of serum; Blank well: Add 100uL of (S0) 1× universal diluent to each well.
4. Incubation: Cover with a sealing film and incubate at 37℃ for 60 minutes.
5. Plate washing: Wash the plate 3 times, and after the last washing, invert the enzyme-linked immunosorbent assay plate and pat off the residual liquid on a clean absorbent paper.
6. Enzyme addition: Calibration well/sample well: Add 100µL of 1×enzyme labeled working solution to each well; Blank hole: Add nothing.
7. Incubation: Cover with a sealing film and incubate at 37℃ for 60 minutes.
8. Plate washing: Wash the plate 5 times, and after the last washing, invert the enzyme-linked immunosorbent assay plate and pat off the residual liquid on a clean absorbent paper.
9. Color development: Add 100uL of color developer to each well and develop color at 37℃ in the dark for 15 minutes.
10. Termination: Add 50µL of termination solution to each well.
11. Measurement: Use an enzyme-linked immunosorbent assay (ELISA) reader to measure the absorbance value at a detection wavelength of 450nm (single wavelength); Alternatively, the absorbance value can be measured using an enzyme-linked immunosorbent assay (ELISA) reader at a detection wavelength of 450/630nm (dual wavelength).

Calculation

1. The dual wavelength detection results do not require zeroing and can be directly fitted with calibration curves and calculated.
2. The following curve fitting methods can be used. Choose the fitting method with the best r value after fitting for result calculation.
3. Four parameter logic (4-P) curve fitting: with concentration value as the horizontal axis and absorbance value as the vertical axis;
4. Polynomial curve fitting: 2nd order polynomial, 3rd order polynomial;
5. Double logarithmic linear fitting: perform linear fitting by taking the logarithm of the concentration value and the logarithm of the absorbance value;
6. Point to point fitting: Linear fitting is performed separately between adjacent points, with segmented calculations;
7. It is recommended to use specialized software for result fitting and calculation, such as ELISACALC, SPSS, Graphpad Prism, etc.
8. The final sample concentration should be multiplied by the dilution factor of the sample.
9. Example graph of curve fitting method, the following data and curves are for illustration only and are not related to the measurement results of this kit.



Limitations of testing

When the sample concentration exceeds the upper limit of the detection range, the dilution factor should be appropriately increased before retesting, and the calculated result should be multiplied by the dilution factor. The sample concentration is below the LOQ quantification limit, and the detection results cannot be accurately quantified.

Product performance indicators

The following results are based on the concentration values of the calibration standard and do not take into account the potential effects of other factors such as matrix effects.

1. Sensitivity: 0.20ng/mL
2. Precision: The intra board precision CV is less than 10% (N=20), and the inter board precision CV is less than 15% (N=20).
3. Analytical specificity: Other related factors were prepared in dilution buffer at a concentration of 10 μ g/mL and no significant cross reactivity was observed.

Note

1. All ingredients in the reagent kit are for research purposes only!
2. The termination solution contains dilute sulfuric acid, which is corrosive and should be handled with caution.
3. The reagent kit contains preservatives, protein components, etc., which may cause skin allergic reactions. Masks should be worn to avoid inhaling light mist.
4. Color developing agents and concentrated washing solutions may cause skin, eye, and respiratory irritation. Masks should be worn to avoid inhaling light mist.
5. Wear protective gloves, protective clothing, eye and facial protection equipment, and wash hands thoroughly after experimental operations.

Technical Points

1. Different batches of kits cannot be mixed. It is not recommended to assemble microporous Flat noodles on different plates into one plate for detection. Although the reagents of the same batch are used, there may be differences between plates.
2. During each experiment, a standard curve should always be prepared for parallel testing, and the previous standard curve should not be used for calculating the results of the next experiment.
3. Do not use expired reagent kits for testing.
4. The substrate color reagent should be colorless. If it turns blue, it indicates that it has deteriorated and cannot be used in experiments.
5. Use appropriate sealing membrane to seal microporous Flat noodles, so that the test results are more reliable.
6. Try to avoid generating bubbles during operation. Do not cross use the caps of different reagent bottles.
7. Experiments should be conducted in accordance with the instructions provided. Failure to follow the instructions may result in changes to the experimental results. Any experimental operations that do not follow the instructions should seek advice from after-sales technical support in advance. Otherwise, we cannot guarantee the reliability of the experimental results.