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Equine Natural Killer Cell (NK) ELISA Kit (quantitative)

Product Number: ELK21813

Shipping and Storage

1. The reagent kit should be stored at 2-8℃ and should not be frozen.
2. Unused microplates should be sealed and stored dry.

Component

Component	Kit Components	48T	96T
Microplate	Pre-coated with capture antibody	8×6 strips	8×12 strips
10× Calibrator	Analyte, 200 ng/mL	100μL	200μL
100× Enzyme Conjugate	100-fold concentrated HRP-labeled detection antibody	50μL	100μL
20× Universal Diluent	Diluent for sample / calibrator / enzyme conjugate	10mL	15mL
20× Concentrated Wash Buffer	20-fold concentrated washing solution	15mL	25mL
Chromogen Solution	TMB, hydrogen peroxide	6mL	12mL
Stop Solution	Dilute sulfuric acid	3mL	6mL

Separate purchase of individual components is available upon request.

Description

This kit adopts the double-antibody sandwich ELISA principle. Capture antibody is pre-coated on the microplate wells to bind the analyte in samples and calibrators. Horseradish peroxidase (HRP)-labeled detection antibody is added to form an immune complex of Antibody-Antigen-Antibody-HRP. TMB chromogen is added; blue color develops if the analyte is present. The reaction is terminated by stop solution. Unbound components are removed by washing. The absorbance (OD value) is measured at 450nm with a microplate reader. The color intensity is positively correlated with the analyte concentration in the sample. The sample concentration is calculated according to the standard curve.

Application

This kit is used for the quantitative detection of equine natural killer cell (NK) antigen concentration in serum, plasma and other samples. For Research Use Only.

Testing Scope

0.312-20ng/mL

Materials required but not supplied

1. Distilled or deionized water, disposable centrifuge tubes, disposable gloves and other consumables
2. Pipettes, multi-channel pipettes and matched tips
3. Beakers, graduated cylinders, reagent bottles
4. Microplate sealing film or alternative sealing material
5. Microplate shaker, centrifuge, vortex mixer and other auxiliary equipment
6. Constant temperature incubator, water bath, microplate reader, microplate washer

Reagent Preparation

1. Preparation of 1× Wash Buffer: If crystals appear in 20×concentrated wash buffer, heat at 37℃ until fully dissolved before use.

Dilute 20-fold with distilled water (1:20): Example: 10mL 20× wash buffer + 190mL distilled water, mix well.

2. Preparation of 1× Universal Diluent: Dilute 20-fold with distilled water (1:20). Example: 10mL 20×universal diluent + 190mL distilled water, mix well.
3. Preparation of 1×Enzyme Conjugate Working Solution: Dilute 100×enzyme conjugate 1:100 with 1×universal diluent. Example: 10μL 100× enzyme conjugate + 990μL 1× universal diluent, mix well. Prepared working solution can be stored at 2–8℃ for 8 hours.
4. Preparation of Working Calibrators: Centrifuge the calibrator vial for 30 seconds before use to collect all liquid at the bottom. Prepare 7 centrifuge tubes: Dilute 10× calibrator 10-fold with 1× universal diluent to prepare S1. Example: 50μL 10× calibrator + 450μL 1× universal diluent to obtain 500μL S1. Add 250μL 1× universal diluent to each of the next 6 tubes, then perform serial two-fold dilution from S1 to prepare S2–S7, total 7 concentration points. 1× universal diluent is used as blank calibrator S0.

Sample Processing Procedure

1. Serum: Collect whole blood with serum collection tubes; let stand at room temperature for natural coagulation, or centrifuge at 1000×g for 15 min to separate serum. Operate gently to avoid hemolysis. Test immediately after collection; if not tested promptly, aliquot and store at ≤ -20℃ for up to 6 months. Freeze-thaw cycles shall not exceed 2 times.
2. Plasma: Collect whole blood with EDTA or heparin anticoagulant tubes. Centrifuge at 1000×g for 15 min to obtain plasma. Avoid hemolysis. Aliquot and store at ≤ -20℃ for up to 6 months; freeze-thaw ≤ 2 times.
3. Tissue: Weigh and mince tissue, add lysis solution at weight/volume ratio 1:4 ~ 1:9 (e.g. 100 mg tissue + 400μL lysis solution). Adjust ratio as needed. Centrifuge homogenate at 5000×g for 5–10 min, collect supernatant for detection.
4. Cell Sample: Suspend appropriate cells, ultrasonically disrupt on ice, centrifuge at 5000×g for 5–10 min, collect supernatant.
5. Cell Culture Supernatant: Centrifuge cell supernatant at 5000×g for 5–10 min, collect supernatant.

Protocol

Equilibrate all reagents to room temperature for about 30 min before use, or warm rapidly at 37℃. Do not open the microplate until fully equilibrated.

1. Layout Setup

Set calibrator wells, sample wells and blank wells; arrange well positions reasonably.

2. Dilution & Loading

2.1. PCalibrator wells: Add 100μL each of S1–S7

2.2. Sample wells: Add 50μL 1× universal diluent first, then 50μL serum sample

2.3. Blank well: Add 100μL 1× universal diluent (S0).

3. Incubation

Seal with plate sealer, incubate at 37℃ for 60 min.

4. Washing

Wash 3 times. After the final wash, invert the plate and pat dry on absorbent paper.

5. Enzyme Addition

Add 100μL 1× enzyme conjugate working solution to calibrator and sample wells; add nothing to blank well.

6. Incubation

Seal again, incubate at 37℃ for 60 min.

7. Washing

Wash 5 times, pat dry residual liquid.

8. Color Development

Add 100μL chromogen solution to each well, incubate at 37℃ in dark for 15 min.

9. Reaction Termination

Add 50μL stop solution to each well.

10. Measurement

Read absorbance at 450nm (single wavelength) or dual wavelength at 450nm / 630nm.

Result Calculation

Dual-wavelength reading requires no blank zeroing; standard curve fitting and calculation can be performed directly.

1. Acceptable curve fitting methods:

2. Quantitative Analysis

1.1. 4-parameter logistic (4-P) fitting

1.2. Polynomial fitting (2nd-order / 3rd-order)

1.3. Double-log linear fitting

1.4. Point-to-point segmental linear fitting

Select the method with the best correlation coefficient (r value).

Professional software such as ELISACALC, SPSS, GraphPad Prism is recommended.

Final sample concentration = calculated value × dilution factor.

Limitations of Assay

If sample concentration exceeds the upper linear range, dilute further and retest, multiply result by dilution factor. Concentrations below the limit of quantitation (LOQ) cannot be accurately quantified.

Performance Characteristics

1. Data derived from calibrator testing, excluding matrix effects.
2. Sensitivity: 0.20 ng/mL
3. Precision: Intra-assay CV < 10% (N=20); Inter-assay CV < 15% (N=20)
4. Analytical Specificity: No obvious cross-reactivity with other related factors at 10 μg/mL in dilution buffer.

Note

1. All kit components are For Research Use Only.
2. Stop solution contains dilute sulfuric acid and is corrosive; handle with caution.
3. Kit components contain preservatives and protein ingredients that may cause skin allergy; wear a mask to avoid inhaling aerosol.
4. Chromogen solution and concentrated wash buffer may irritate skin, eyes and respiratory tract; use protective mask.
5. Wear gloves, lab coat and eye/face protection; wash hands thoroughly after operation.

Technical Notes

1. Do not mix components from different batch numbers; do not assemble strips from different plates even within the same batch.
2. Run a fresh standard curve in each assay; do not reuse previous standard curves.
3. Do not use expired kits.
4. Fresh chromogen solution should be colorless; discard if turned blue.
5. Use qualified plate sealing film to ensure reliable results.
6. Avoid bubble formation during pipetting; do not cross-use bottle caps.
7. Strictly follow the manual procedure. Any deviation without technical support consultation is not guaranteed for result reliability.