ab313908 – Human NK-p44 ELISA Kit

For the quantitative measurement of NK-p44 in human serum, plasma, and cell culture supernatants.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab313908

Storage and Stability:

The entire ELISA kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. Observe the storage conditions for individual prepared components in the Reagent Preparation

Materials Supplied

Item	Quantity	Storage Condition
500X HRP-Streptavidin	200 µL	-20°C
20X Wash Buffer	25 mL	-20°C
5X Assay Diluent B (Item E)	15 mL	-20°C
5X Assay Diluent D (Item K)	15 mL	-20°C
Stop Solution; 0.2M Sulfuric Acid	8 mL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Human NK-p44 Antibody-coated ELISA Plate	1 unit	-20°C
Lyophilized Human NK-p44 Protein Standard	2 vials	-20°C
Biotinylated Human NK-p44 Detection Antibody	2 vials	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µl to 1 ml volumes.
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard or sample dilutions

Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

5x Assay Diluent: Dilute 5X Assay Diluent D (Item K) and 5X Assay Diluent B (Item E) 5-fold with deionized or distilled water before use. 1X Assay Diluent D (Item K) should be used to dilute serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-fold.

Note: Levels of NK-p44 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

Biotinylated Human NK-p44 Detection Antibody: Briefly spin the Detection Antibody vial before use. Add 100 µl of 1X Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B (Item E).

20X Wash Buffer (Item B): If the 20X Wash Buffer contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

HRP-Streptavidin Concentrate: Briefly spin the 500X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 500-fold with 1X Assay Diluent B (Item E).

For example: Briefly spin the vial and pipette up and down to mix gently. Add 20 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1X Assay Diluent B to prepare a 500-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Standard Preparation

Always prepare a fresh set of standards for every use.
Discard working standard dilutions after use as they do not store well.
The following section describes the preparation of a standard curve for duplicate measurements (recommended).

- Briefly spin the Lyophilized Human NK-p44 Protein Standard vial.
- Add 400 µl 1X Assay Diluent D (Item K, Assay Diluent D should be diluted 5-fold with deionized or distilled water before use) into the vial to prepare a 15 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix.
- Add 100 µl of NK-p44 standard solution from the vial, into a tube with 400 µl 1X Assay Diluent D (Item K) to prepare a 3000 pg/ml standard solution.
- Pipette 300 µl 1X Assay Diluent D (Item K) into each tube.
- Use the 3000 pg/ml standard solution to produce a dilution series (shown below). Adding 200 µL from Tube #1 to #2, then from #2 to #3, etc
- Mix each tube thoroughly before the next transfer.
- 1X Assay Diluent D (Item K) serves as the zero standard (0 pg/ml).

Tube #	Volume to dilute	Volume of 1X Assay Diluent	Final Concentration pg/mL
1	3000 pg/mL standard stock solution	---	3000
2	200 µL of tube #1	300 µL	1200
3	200 µL of tube #2	300 µL	480
4	200 µL of tube #3	300 µL	192
5	200 µL of tube #4	300 µL	76.8
6	200 µL of tube #5	300 µL	30.72
7	200 µL of tube #6	300 µL	12.29
8	--	300 µL	0

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use. We recommend that you assay all standards, controls, and samples in duplicate. Prepare all reagents, working standards, and samples as directed in the previous sections. **Note:** 1X Assay Diluent should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-fold.

1. Label removable 8-well strips as appropriate for your experiment.

2. Add 100 µL of standard or sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature with gentle shaking.

3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multichannel Pipette or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µL of 1X prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 µL of prepared Streptavidin solution each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 3.

8. Add 100 µL of TMB Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

Calculation of results

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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