ClinMax™ Human NKp46/NCR1 ELISA Kit

Catalog Number：CEA-B040

Assay Tests: 96 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing your experiment.

Product information

This kit is specifically designed for the accurate quantitation of human Natural Cytotoxicity Triggering Receptor 1 (NKp46/NCR1) from cell culture supernates, serum and plasma.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples are added to the wells. After the removal of any unbound materials through washing, biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

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NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

Manufactured and distributed by
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ACROBiosystems Inc.
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Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA040-B01	Pre-coated Anti-NKp46 Antibody Microplate	1 plate
CEA040-B02	Human NKp46 Standard	20 μg×2
CEA040-B03	Biotin-Anti- NKp46 Antibody Con. Solution	200 μL
CEA040-B04	2×Biotin-Antibody Dilution Buffer	8 mL
CEA040-B05	Streptavidin-HRP Con. Solution	500 μL×2
CEA040-B06	Streptavidin-HRP Dilution Buffer	15 mL
CEA040-B07	20× Washing Buffer	50 mL
CEA040-B08	Sample Dilution Buffer	15 mL×2
CEA040-B09	Substrate Solution	12 mL
CEA040-B10	Stop Solution	6 mL

Storage

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date.
For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

Contents	Storage conditions
Pre-coated Anti-NKp46 Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C.
Human NKp46 Standard	Aliquot and store for up to 1 month at -70°C in a freezer. Avoid repeated freeze-thaw cycles.

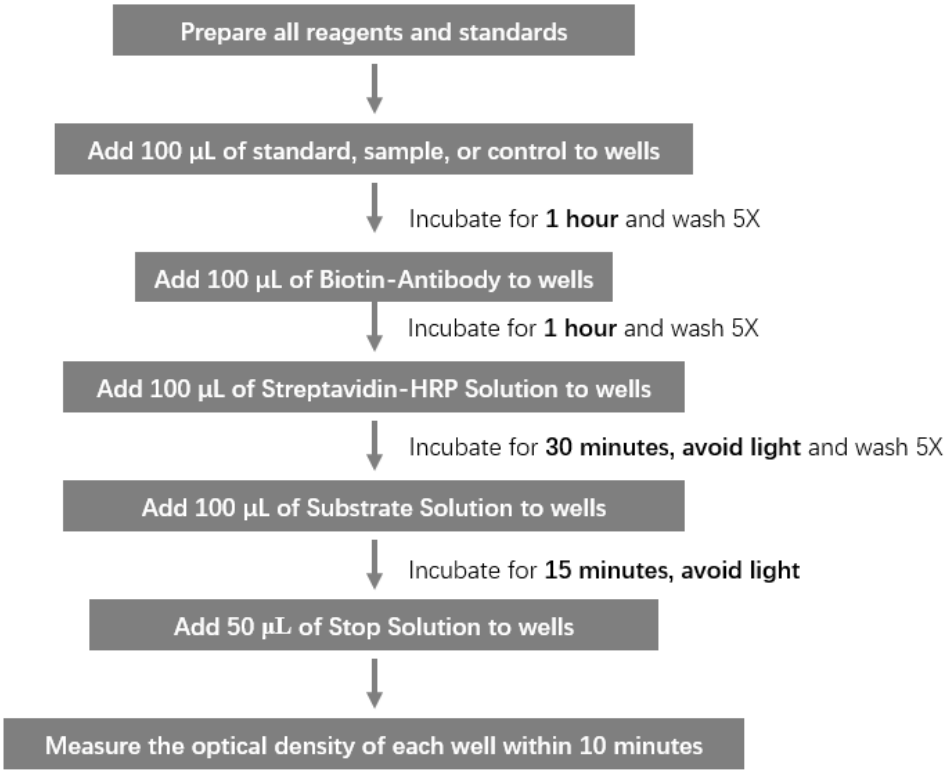
NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

Required materials not supplied.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure or distilled water
Consumables	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

Workflow

Analyte: NKp46



NOTE: Incubation temperature is 18 ℃-25 ℃

Prepare the working buffers and standard dilutions.

IMPORTANT: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. 1×Biotin-Antibody Dilution Buffer： Dilute 8 mL 2×Biotin-Antibody Dilution Buffer with deionized or distilled water to 16 mL.
3. Biotin-Anti-NKp46 Antibody Solution: Add 120 µL of Biotin-Anti-NKp46 Antibody Con. Solution to 12 mL 1×Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
4. NKp46 Streptavidin-HRP Solution: Add 600 µL of NKp46 Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

Prepare the reconstituted standard.

Add 100 µL ultrapure water to the provided lyophilized product（Catalog: CEA040-B02）, dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted human NKp46 Standard is 200 µg /mL.

NOTE: *Avoiding vigorous shaking or vortexing. The reconstituted solution should be stored at -70°C. The freeze-thaw cycle should not exceed 1 time, and the size of the aliquot should not be less than 10 µg.*

Prepare the standard serial dilutions.

1. Label a tube "**Cm**". Add 5 µL of the reconstituted human NKp46 Standard and 1995 µL of Sample Dilution Buffer to tube Cm, gently mix well.
2. Label 7 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
3. Add 6 µL of the liquid from **Cm** and 2994 µL of Sample Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 =1000 pg/mL).
4. Prepare serial dilutions for the standard curve as follows: Add 500 µL of Sample Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).
5. Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 500 pg/mL).
6. Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7.
7. Sample Dilution Buffer serves as zero standard (blank).

PROCEDURE OF ASSAY

1. Add 100 µL of NKp46 Standard, sample, or control to wells. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hours**.
2. Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
3. Add 100 µL Biotin-Anti-NKp46 Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hours.**
4. Repeat step 2.
5. Add 100 µL of NKp46 Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for 3**0 minutes, avoid light.**
6. Repeat step 2.
7. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
8. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.
   Note: *the color in the wells should change from blue to yellow.*
9. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes.
   Note: *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

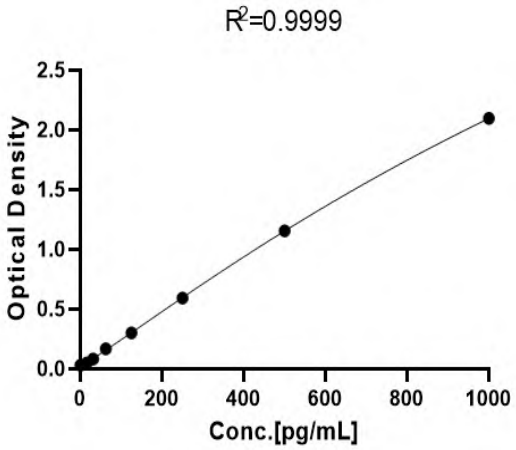
CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample. Then, subtract the average optical density (O.D.) of the zero standard(blank).
2. Establish a standard curve by processing the data using computer software capable of executing a four-parameter logistic (4-PL) curve fitting.
3. Normal range of Standard curve: $R^2 \geqslant 0.9900$.
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

Typical data

Note: *For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.*

NKp46 Standard (pg/mL)	$OD_{450nm-630nm}$
1000	2.098
500	1.157
250	0.595
125	0.304
62.5	0.170
31.25	0.084
15.625	0.051
Blank	0.033



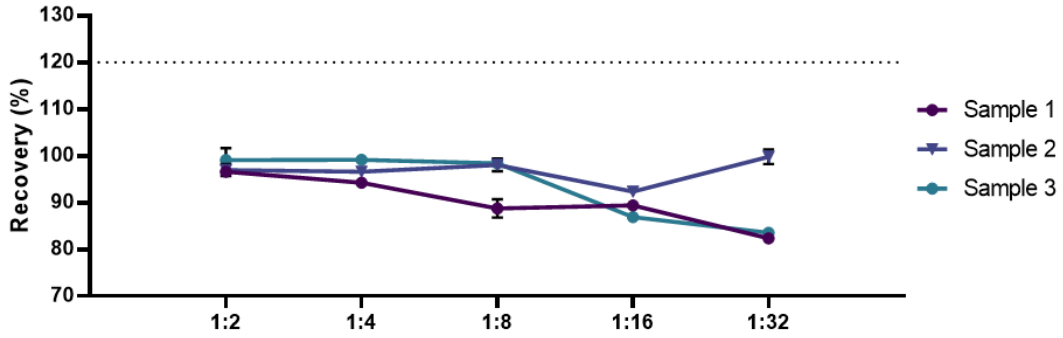
PERFORMANCE CHARACTERISTICS

1. Sensitivity

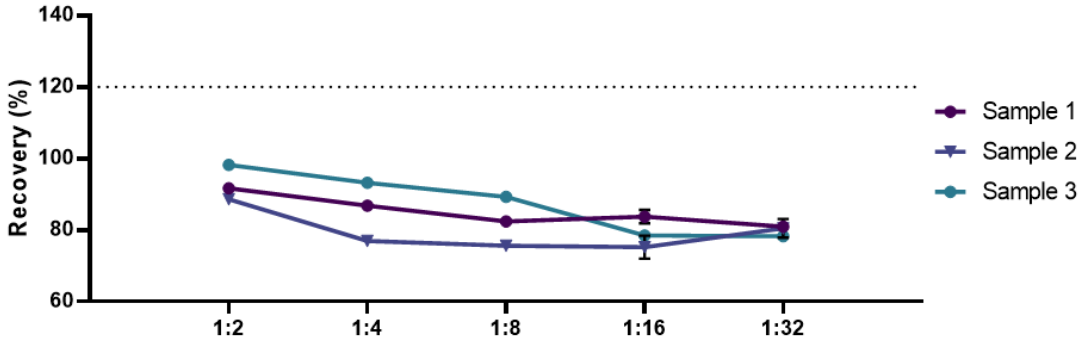
The minimum detectable concentration (MDC) of NKp46 is typically less than 15.0 pg/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

2. Linearity

Three samples (Serum) spiked with high concentrations of NKp46 were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of NKp46 for serum samples is 93.5%.



Three samples (EDTA plasma) spiked with high concentrations of NKp46 were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of NKp46 for serum samples is 84.0%.



3. Intra-Assay Precision

Ten replicates of each of 4 samples containing different NKp46 concentrations were tested in one assay. Acceptable criteria: CV < 10%.

Sample Concentration (pg/mL)	Mean (pg /mL)	SD	Numbers	CV
1000	913.6	52.8	10	5.8%
750	739.9	71.2	10	9.6%
500	504.8	42.0	10	8.3%
31.25	30.7	2.8	10	9.1%

4. Inter-Assay Precision

Five samples containing different concentrations of NKp46 were tested in independent assays. Acceptable criteria: CV<15%.

Sample Concentration (pg/mL)	Mean (pg/mL))	SD	Numbers	CV
1000	927.5	52.1	9	5.6%
750	739.9	76.1	9	10.3%
500	505.7	36.2	9	7.2%
31.25	30.4	2.9	9	9.5%
15.625	16.1	1.3	9	7.8%

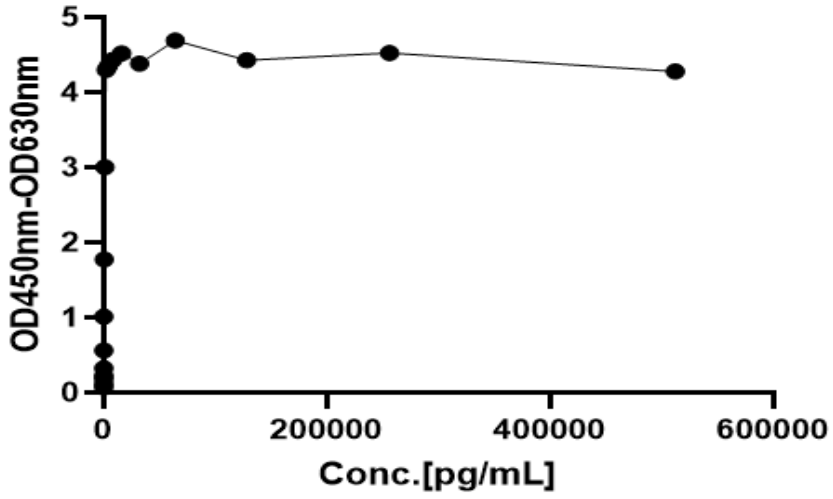
5. Recovery

Recombinant NKp46 was spiked into 3 human serum samples, and then analyzed. The average recovery of NKp46 for serum samples is 97.8%.

Sample ID	Conc Measured (pg/mL)	Conc Added (pg/mL)	Conc Recovered (pg/mL)	Recovery
1	782.0	750	679.3	91.9%
	574.6	500	471.9	96.4%
	353.2	250	250.5	104.3%
2	883.5	750	773.7	104.6%
	647.4	500	537.7	109.7%
	338.7	250	228.9	96.0%
3	752.4	750	630.3	85.7%
	575.8	500	453.7	93.2%
	355.6	250	233.5	98.3%

6. Hook Effect

Not be affected by the concentration of NKp46 up to 16 ng/ml.



7. Interference Effect

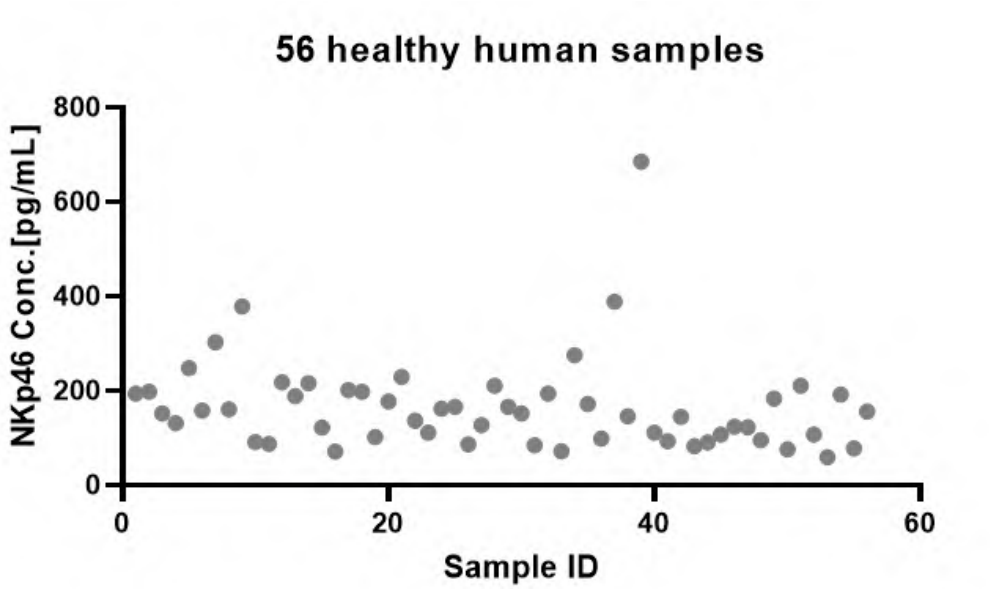
To evaluate the hemolysis matrix effect and high-dose triglyceride matrix effect of assay, serum samples spiked with high concentrations of hemoglobin (2%), or triglyceride (3 mg/mL) were tested. Results shown that all spiked analytes had recoveries between 88% and 112%, no hemolysis matrix effect and high-dose triglyceride matrix effect was observed in assay.

Spiked Material	ID	Conc-1(pg/mL)	Conc-2(pg/mL)	Mean(pg/mL)	Recovery
2% Hemoglobin (v/v)	Sample 1	684.91	680.32	682.62	93%
	Spiked Sample1	653.95	621.70	637.83	
	Sample 2	388.46	378.19	383.33	112%
	Spiked Sample 2	431.82	426.89	429.36	
	Sample 3	201.30	197.40	199.35	103%
	Spiked Sample 3	205.34	206.71	206.03	
	Sample 4	90.95	86.70	88.82	94%
	Spiked Sample 4	87.62	80.24	83.93	

Spiked material	ID	Conc-1 ((pg/mL)	Conc-2 (pg/mL)	Mean (pg/mL)	Recovery
Triglyceride (3 mg/mL)	Sample 1	684.91	680.32	682.62	102%
	Spiked Sample1	690.12	696.03	693.08	
	Sample 2	388.46	378.19	383.33	91%
	Spiked Sample 2	359.96	337.45	348.71	
	Sample 3	201.30	197.40	199.35	92%
	Spiked Sample 3	181.89	183.12	182.51	
	Sample 4	90.95	86.70	88.82	88%
	Spiked Sample 4	78.93	77.36	78.15	

8. Sample Values

56 healthy serum samples were evaluated for the concentrations of human NKp46 in assay.



9. Specificity

No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines at up to 1 μg/mL.

Human	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-12 p70, IL-10, IL-13, IL-15, IL-17, GM-CSF, TNF-α

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts