ab283983 – Human CNDP2/CN2 ELISA Kit

For the quantitative measurement of CNDP2/CN2 in Human plasma and serum samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab283983

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the Standard, SP Conjugate, and Biotinylated Antibody which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Human CNDP2/CN2 Microplate	96 wells	4°C
100X Streptavidin-Peroxidase (SP) Conjugate	80 µL	-20°C
10X Diluent M Concentrate	20 mL	4°C
1X Standard Diluent	2 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Chromogen Substrate	7 mL	4°C
CNDP2/CN2 Standard	1 vial	-20°C
Sealing Tapes	3 units	4°C
Stop Solution	11 mL	4°C
40X Biotinylated Human CNDP2/CN2 Antibody	1 vial	-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
Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel)
Deionized or distilled reagent grade water

Reagents Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
Prepare only as much reagent as is needed on the day of the experiment.
If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
Δ Note: Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

10X Diluent M Concentrate: Dilute the Diluent M Concentrate 10- fold with reagent grade water to produce a 1X solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Store for up to 30 days at 4°C

40X Biotinylated Human CNDP2/CN2 Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 40-fold with Diluent M to produce a 1X solution. The undiluted antibody should be stored at -20°C

20X Wash Buffer Concentrate: Dilute the Wash Buffer Concentrate 20- fold with reagent grade water to produce a 1X solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle.

100X SP Conjugate: Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with Diluent M to produce a 1X solution. The undiluted conjugate should be stored at -20°C.

Standard Preparation

Always prepare a fresh set of standards for every use.
Prepare serially diluted standards immediately prior to use.
Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
The following section describes the preparation of a standard curve for duplicate measurements (recommended).
Reconstitution of the CNDP2/CN2 Standard vial to prepare a 200 ng/mL Stock Standard.

- First consult the CNDP2/CN2 Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of 1X standard Diluent to add when resuspending the CNDP/CN2 Standard vial to produce a 200 ng/mL ng/mL Stock Standard by using the following equation:
    - C_S = Starting mass of CNDP2/CN2 Standard (see vial label) (ng)
    - C_F = The 200 ng/mL CNDP2/CN2 Stock Standard final required concentration
    - V_D = Required volume of 1X Standard Diluent for reconstitution (µL)
    - Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example: **Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided**.

CS = 160 ng of CNDP2/CN2 Standard in vial

CF = 200 ng/mL CNDP2/CN2 Standard #1 final concentration

VD = Required volume of 1X Standard Diluent for reconstitution (160 ng / 200 ng/mL) x 1,000 = 800 µL

- Reconstitute the CNDP2/CN2 Standard vial by adding the appropriate calculated amount VD of 1X Standard Diluent to the vial to generate the 200 ng/mL CNDP2/CN2 Standard #1. Mix gently and thoroughly.
- Allow the reconstituted 200 ng/mL CNDP2/CN2 #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label eight tubes #1 – 8.
- Add 120 µL of 1X Diluent M to tube #1 – 8.
- To prepare Standard #1, add 120 µL of the Stock Standard into tube #1 and mix gently.
- To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently
- Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent M serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume 1X Diluent M (µL)	CNDP/CN2 (ng/mL)
1	120 µL Stock standard (200 ng/mL)	120 µL	100
2	120 µL standard #1	120 µL	50
3	120 µL standard #2	120 µL	25
4	120 µL standard #3	120 µL	12.5
5	120 µL standard #4	120 µL	6.25
6	120 µL standard #5	120 µL	3.125
7	120 µL standard #6	120 µL	1.563
8	-	120 µL	0.0

Sample Preparation

Plasma

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant.
Centrifuge samples at 3000 x g for 10 minutes and collect plasma.
The sample is suggested for use at 1X; however, user should determine optimal dilution factor depending on application needs.
The undiluted samples can be stored at -20°C or below for up to 3 months.
Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

Serum

Samples should be collected into a serum separator tube.
After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum.
The sample is suggested for use at 1X; however, user should determine optimal dilution factor depending on application needs.
The undiluted samples can be stored at -20°C or below for up to 3 months.
Avoid repeated freeze-thaw cycles

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

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

1. Add 50 µL of Human CNDP2/CN2 Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
2. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; tap 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µL of Wash Buffer per well. Invert the plate each time and decant the contents; tap 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µL of Wash Buffer per well; invert the plate and tap 4-5 times on absorbent material to completely remove the liquid.
3. Add 50 µL of Biotinylated Human CNDP2 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours.
4. Wash the microplate as described above.
5. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance
6. Wash the microplate as described above.
7. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 20 minutes or until the optimal blue color density develops.
8. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles formed.
9. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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Additional information

CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

PERFORMANCE CHARACTERISTICS

1. This assay recognizes both natural and recombinant human CNDP2.
2. The minimum detectable dose of human CNDP2 as calculated by 2SD from the mean of a zero standard was established to be 0.92 ng/mL
3. Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
4. Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	5.8	10.6

RECOVERY

Standard Added Value	3.125 – 25 ng/mL
Recovery %	89 – 112%
Average Recovery %	98%

LINEARITY

Average Percentage of Expected Value (%)		
Sample Dilution	**Plasma**	**Serum**
1x	92%	89%
2x	98%	95%
4x	105%	110%

CROSS REACTIVITY

Species	Cross-Reactivity (%)
Canine	15%
Bovine	None
Wquine	15%
Monkey	70%
Mouse	15%
Rat	50%
Swine	30%
Rabbit	None

Protein	Cross-Reactivity (%)
Human Cystathionase (CTH)	None

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide
For all technical or commercial enquiries please go to:
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