



User's Manual

Human CNPY2 ELISA Kit

REF DEIASL603

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

General Description

CNPY2, also named as MSAP, TMEM4 and ZSIG9, is a positive regulator of neurite outgrowth (PMID: 12826659). It interacts with the ezrin-radixin-moesin (ERM)-like myosin regulatory light chain-interacting protein (MIR), and the two proteins are co-localized in cell lines and in primary neurons. CNPY2 is a HIF-1 α regulated, secreted angiogenic growth factor that promotes smooth muscle cells migration, proliferation and tissue revascularization (PMID: 25589425). CNPY2 enhances cell spreading in fibroblasts and migration of rat C6 glioma cells (PMID: 15905959). CNPY2 was also shown to influence the expression of low-density lipoprotein receptor and FGF21 (PMID: 22378787). This kit is used to quantify CNPY2 level in vivo.

Reagents And Materials Provided

1. Microplate –Capture antibody-coated 96-well microplate (8 wells x 12 strips): 1 plate. Store at 2–8°C
2. Standard – Lyophilized: 2 Bottles. Store at 2–8°C.
3. Detection Antibody (100X) – 120 μ L/vial: 1 Vial. Store at 2–8°C.
4. HRP-Conjugated Antibody (100X) – 120 μ L/vial Or Streptavidin-HRP (100X) – 120 μ L/vial*: 1 Vial. Store at 2–8°C.
5. Sample Diluent – 30 mL/bottle: 1 Bottle. Store at 2–8°C.
6. Detection Diluent – 30 mL/bottle: 1 Bottle. Store at 2–8°C.
7. Extraction Reagent** – 30 mL/bottle: 1 Bottle. Store at 2–8°C.
8. Washing Buffer Concentrate (20X) – 30 mL/bottle: 1 Bottle. Store at 2–8°C.
9. Tetramethylbenzidine Substrate (TMB) – 12 mL/bottle: 1 Bottle. Store at 2–8°C.
10. Stop Solution – 12 mL/bottle: 1 Bottle. Store at 2–8°C.
11. Plate Cover Seals: 3 Pieces

* Kit-dependent, specific data can be found in individual kit datasheets.

** Only required when working with cell lysates and homogenized tissue samples.

Please Note:

- 1) Do not use the kit after the expiration date.
- 2) Sample diluent is designed for dilution of standard and samples. Please use appropriate sample diluent based on the sample type.
- 3) Detection diluent is designed for dilution of detection antibody and HRP-conjugated antibody.

Materials Required But Not Supplied

1. A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
2. Calibrated, adjustable precision pipettes and disposable plastic tips. (A manifold multi-channel pipette is recommended for large assays.)

3. Distilled, deionized water.
4. Plate washer: automated or manual.
5. Glass or plastic tubes to prepare standard and sample dilutions.
6. Absorbent paper towels.
7. Beakers and graduated cylinders.
8. 100 mM PMSF (phenylmethanesulfonyl fluoride) stock solution (1g PMSF in 57 mL isopropyl alcohol).
9. Log-log or semi-log graph paper or computer and software for ELISA data analysis (a four-parameter logistic (4-PL) curve-fit is recommended).

Storage

All the reagents are stored at 2-8°C for 6 months or -20°C for 12 months. Refer to the protocol for further storage instructions.

Specimen Collection And Preparation

Please Note:

- 1) All biological samples should be carried out in accordance with institutional safety guidelines and restrictions.
- 2) Please run a preliminary experiment to optimize the sample dilution and to check if the sample is within the assay range.

A. Serum

Allow blood samples to clot for 30 minutes prior to centrifugation for 15 minutes at 1000 x g. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

B. Plasma

Use EDTA or heparin as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

Please Note: Do not use turbid or grossly hemolyzed samples. Mix thawed samples thoroughly before starting the ELISA assay.

C. Cell culture supernatants

Pellet cells and remove particulates by centrifugation and assay immediately, or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

D. Cell lysates

1. Pellet cells and wash by centrifuging at 5000 x g for 10 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times.
2. Count cells then repellet (5000 x g for 10 minutes).
3. Add PMSF to the Extraction Reagent to a final concentration of 1 mM immediately prior to performing cell lysis.
4. Per 10^7 cells add 1 mL of Extraction reagent (containing PMSF 1 mM). Incubate cell suspension on ice for

30 minutes; be sure to invert tube containing the cell suspension several times to complete lysis.

5. Centrifuge cell lysate at 10,000 x g for 10 minutes at 4°C.
6. Measure the concentration of total protein in cell lysate using desired method of protein concentration assay.
7. Where possible, keep samples on ice to avoid protein degradation.

E. Tissue homogenates

1. Add PMSF to the Extraction Reagent to a final concentration of 1 mM immediately prior to performing cell lysis.
2. Add 1 mL of Extraction Reagent containing PMSF per 100 mg tissue. Invert tube containing the tissue and Extraction Reagent several times while keeping on ice for 30 minutes to ensure complete lysis.
3. Homogenize the tissue completely using desired method on ice.
4. Centrifuge tissue homogenates at 10,000 x g for 10 minutes at 4°C.
5. Retain the supernatant and store at -80°C.
6. Measure the concentration of total protein in tissue homogenates using desired method of protein concentration assay.
7. Avoid protein degradation by performing all the above procedures on ice where possible.

F. Urine

Centrifuge urine samples for 20 minutes at 1000 x g. Remove particulates and assay immediately or aliquot and store samples at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Reagent Preparation

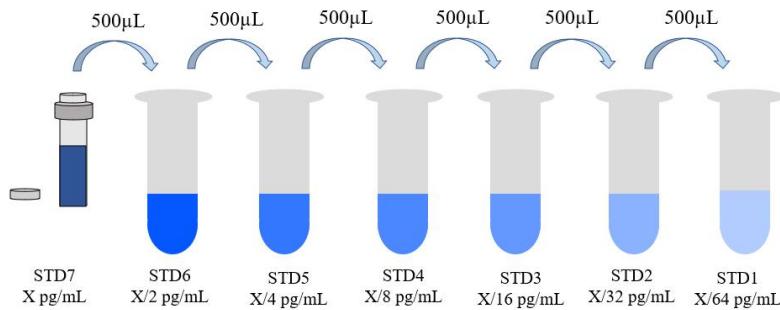
Please Note:

- 1) Prior to use, equilibrate all reagents at room temperature for 20 to 30 minutes. (Standard, Detection antibody and HRP-conjugated antibody can be used immediately since only a small amount of each is needed.)
- 2) Please read this entire section thoroughly prior to carrying out the assay.

A. Standard

Always prepare a fresh set of standards for every use. It is recommended to run the experiment in duplicate at a minimum.

1. Reconstitute the Standard with 2 mL* Sample Diluent (label it "sd7"). This reconstitution gives a stock solution with an assumed concentration of X pg/mL. Use Standards within 30 minutes of reconstitution. *kit-dependent, specific data can be found in individual kit datasheets.
2. Perform serial dilutions of standards as described in the following figure; i.e., carry over 500 µL of standard from last dilution into a tube containing 500 µL fresh Sample Diluent. Mix thoroughly between steps.
3. Use Sample Diluent as your blank/zero standard; label it "sd0".



X pg/mL * an individual standard concentration and a serial dilution flow chart can be found in the corresponding kit datasheet.

	Add # μ L of Standard diluted in the previous step	# μ L of Sample Dilution
“STD7”	-	2000 μ L**
“STD6”	500 μ L	500 μ L
“STD5”	500 μ L	500 μ L
“STD4”	500 μ L	500 μ L
“STD3”	500 μ L	500 μ L
“STD2”	500 μ L	500 μ L
“STD1”	500 μ L	500 μ L

**Kit-dependent, specific data can be found in individual kit datasheets.

B. Detection Antibody

1. Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μ L 100X Detection Antibody + 990 μ L Detection Diluent.

Please Note: Centrifuge the 100X Detection Antibody solution for a few seconds prior to use.

C. HRP-conjugated Antibody

1. Dilute 100X HRP-conjugated antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μ L 100X HRP-conjugated antibody + 990 μ L Detection Diluent.

Please Note: Centrifuge the 100X HRP-conjugated antibody solution for a few seconds prior to use.

D. Wash Buffer

1. Allow the 20X Wash Buffer to reach room temperature before use. Dilute entire 30 mL of 20X Wash Buffer concentrate with 570 mL deionized, distilled water. If crystals remain in the concentrate, warm to 37°C and mix gently until the crystals have dissolved completely. Store at 2–8°C.

Assay Procedure

Please Note:

- 1) Equilibrate all reagents and samples at room temperature before use.
- 2) Gently mix each reagent before use.
- 3) It is recommended to assay all standards, controls, and samples in duplicate.
- 4) A standard curve must be run for each assay.

1. Prepare all reagents, samples, and working standards as instructed. (See sections "Sample Preparation" and "Reagent Preparation" (including Standard Preparation)).
2. Take out the required number of microplate strips and place the microwells in the strip holder. In the meantime, return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used as soon as possible.
3. Add 100 µL of each standard and sample to the appropriate wells. (Make sure sample addition is uninterrupted and completed within 5 to 10 minutes).
4. Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 1 or 2 hours* at 37°C in a humid environment. (See individual kit datasheet for specific incubation times).

*optimal conditions are provided with individual product datasheet.

5. Wash wells:
 - i. Gently remove the cover seal.
 - ii. Discard the liquid from wells by aspirating or decanting.
 - iii. Remove any residual solution by tapping the plate a few times on fresh paper towels.
 - iv. Wash 4 times with 1X Wash Buffer, using at least 300 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.
6. Add 100 µL of 1X Detection Antibody solution (see section "Reagent Preparation") to each well. Seal plate with cover seal and incubate for 1 hour at 37°C in a humid environment. Repeat the washes in step 5.
7. Add 100 µL of 1X HRP-conjugated antibody to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C in a humid environment. Repeat the washes in step 5.
8. Signal development: Add 100 µL of TMB substrate solution to each well. Incubate for 15 to 20 minutes at 37°C in the dark. A positive reaction will be indicated by the color blue. (Longer incubation times are recommended in the event of the blue color appearing too pale).
9. Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. The color will change from blue to yellow. Please Note: Avoid any skin and eye contact with Stop solution.
10. Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm). The absorbance should be measurable immediately after adding Stop solution. DO NOT exceed 5 minutes.

Calculation

Average the duplicate readings for each standard and sample and subtract the average zero standard absorbance (obtained from the average of the "sd0" readings). The best-fit standard curve can be determined by regression analysis using four-parameter logistic curvefit (4-PL). As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. The data may be linearized by plotting the log of the Standard concentrations versus the log of the OD readouts. The best-fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Please Note: If the samples have been diluted, the OD readout from the standard curve must be multiplied by the corresponding dilution factor.

Precision

Intra-Assay

SAMPLE	N	MEAN (ng/mL)	SD	CV%
1	20	0.96	0.08	8.2
2	20	1.83	0.11	6.2
3	20	6.17	0.50	8.0

Inter-Assay

SAMPLE	N	MEAN (ng/mL)	SD	CV%
1	24	0.61	0.05	8.7
2	24	1.74	0.17	9.5
3	24	5.20	0.47	9.1

Detection Range

0.156-10 ng/mL

Sensitivity

0.01 ng/mL

Recovery

SAMPLE TYPE	AVERAGE	RANGE
Human plasma	96%	85%-114%
Cell lysates	88%	85%-91%
Cell culture supernatants	88%	80%-95%

Precautions

1. Do not use the kit after the expiration date.
2. This product is for research use only and is not for use in human or animal therapeutic or diagnostic use.
3. Strictly adhere to the instructions in this manual. Do not mix or substitute reagents or materials from other kit lots or other sources.
4. Samples may require further dilution if the readout values are higher than the highest standard OD reading. Dilute the samples with Sample Diluent and ensure that their absorbance reading falls within those generated by the standards.
5. Perform the assay in replicates as errors can be introduced by the operator, such as poor pipetting technique, wash technique, or incorrect incubation times or temperatures.