HUMAN CONNECTIVE TISSUE-ACTIVATING PEPTIDE III (CTAP III) / NEUTROPHIL ACTIVATING PROTEIN-2 (NAP-2) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN NAP-2 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA

PRODUCT INFORMATION:

ELISA NAME	HUMAN NAP-2 ELISA
Catalog No.	SK00434-01
Lot No.	
Formulation	96 T
Standard range	31.25-2000 pg/mL
Sensitivity	15 pg/mL
Sample require	100 µL
Dilution Factor	>1000 for serum or plasma (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, Plasma, Cell Culture supernates
Specificity	Human CTAP III / NAP-2
Calibration	Human NAP-2 recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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DESCRIPTION

This Human NAP-2 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human NAP-2 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human NAP-2 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural NAP-2 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human NAP-2. The capture antibody can bind to the human NAP-2 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human NAP-2 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human NAP-2 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
NAP-2 Microplate - 96 well microplate coated with an antibody specific for human NAP-2.	**434-01-01**	**1 plate**
NAP-2 Standard – 1000 pg/vial of lyophilized recombinant human NAP-2.	**434-01-02**	**2 vials**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of lyophilized biotinylated antibody against human NAP-2.	**434-01-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant human NAP-2.	**434-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered solution with preservative.	**DB01**	**2 bottles**
HRP Diluent Solution – 12 mL of buffered solution with preservative.	**DB06**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution – 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin- HRP Conjugate 100-fold concentrated solution and other

components may be stored at 2 - 8° C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Centrifuge and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

SAMPLE PREPARATION

Serum and Plasma samples may require a 1000-fold or greater dilution. A suggested 10-fold dilution is 10 µL sample + 90 µL Dilution Buffer. A suggested 100-fold dilution is 10 µL of 10-fold diluted sample + 90 µL Dilution Buffer. A suggested 1000-fold dilution is 25 µL of 100-fold diluted sample + 225 µL Dilution Buffer.
Optimal dilutions should be determined by each laboratory for each application with a pretest.
Use polypropylene test tubes.

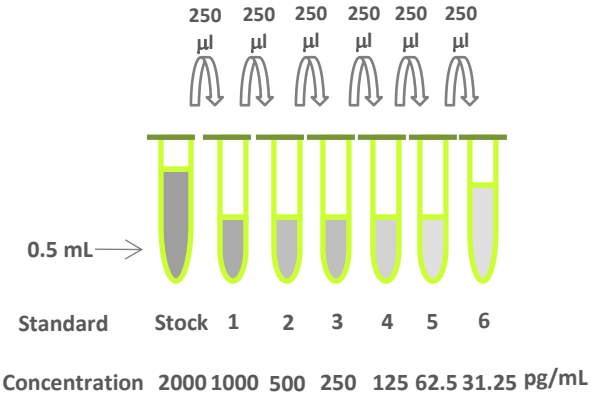
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

NAP-2 Standard - Reconstitute the NAP-2 standard with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	0.5 mL	2000 pg/mL
# 1	250µL of stock	250µL	1000 pg/mL
# 2	250µL of 1	250µL	500 pg/mL
# 3	250µL of 2	250µL	250 pg/mL
# 4	250µL of 3	250µL	125 pg/mL
# 5	250µL of 4	250µL	62.5 pg/mL
# 6	250µL of 5	250µL	31.25 pg/mL



Positive Control - Reconstitute the Positive Control with 0.5 mL of Dilution Buffer. *Positive Control could be reused within a few days if stored at -20° C or -70° C.*

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HPR Diluent Solution (DB06) i**nto a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. *Note: 1x working solution of Streptavidin-HRP Conjugate could be used within a few days(protect from light).*

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **Dilution Buffer** to Blank wells.
4. Add 100 µL per well of **Standard dilutions**, **sample,** or **positive control**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to 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.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 10-20 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 2000 pg/mL may result in inaccurate, low human NAP-2 levels. Such samples require further external predilution according to expected human NAP-2 values with Dilution Buffer in order to precisely quantify the actual human NAP-2 level.

SPECIFICITY

This assay recognizes both natural and recombinant human NAP-2. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rh NAP-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEIN NAME	CROSS-REACTIVITY
Human NAP-2	100%
Human GRO-α	0
Human IL-8	0
Human MCP-1	0
Human SDF-1α	0
Human SDF-1β	0

TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 NM (CORRECTED)
Blank	0 (0.135)
31.25	0.016
62.5	0.027
125	0.057
250	0.123
500	0.330
1000	0.929
2000	2.133

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard dilutions, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptavidin HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 10-20 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.