

HUMAN NOV/CCN3 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN NOV/CCN3 CONCENTRATIONS
IN SERUM, PLASMA AND CELL CULTURE OR
TISSUES



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

PURCHASE INFORMATION:

ELISA NAME	HUMAN NOV/CCN3 ELISA
Catalog No.	SK00265-01
Lot No.	
Formulation	96 T
Standard range	31.25 – 2000pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µl
Sample Type	Serum, plasma, cell culture or tissue
Specificity	Human NOV/CCN3 only
Dilution Factor	10 (Optimal dilutions should be determined by each laboratory for each application)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2°C - 8°C

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INTRODUCTION

Human NOV/CCN3 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human NOV/CCN3 in cell culture supernates, serum, and plasma. It contains recombinant human NOV/CCN3 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human NOV/CCN3. Results obtained with naturally occurring NOV/CCN3 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human NOV/CCN3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for NOV/CCN3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any NOV/CCN3 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for NOV/CCN3 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of NOV/CCN3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- _ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- _ The kit should not be used beyond the expiration date on the kit label.
- _ Do not mix or substitute 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.
- _ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
- _ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- _ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all

factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
NOV/CCN3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against NOV/CCN3.	265-01-01	1 plate
NOV/CCN3 Standard – 2000 pg/vial of recombinant human NOV/CCN3 in a buffered protein base with preservatives; lyophilized.	265-01-02	1 vial
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of Biotinylated polyclonal antibody against NOV/CCN3 with preservatives; lyophilized.	265-01-03	1 vial
Positive Control – one vial of recombinant human NOV/CCN3, lyophilized	265-01-04	1 vial
Streptavidin-HRP Conjugate – 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugated to HRP	SAHRP	1 vial
Dilution Buffer – 60mL/bottle of buffered protein based solution with preservatives	DB01	1 bottle
Wash Buffer – 50 mL/bottle, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution – 11 mL/bottle of TMB substrate solution	TMB01	1 bottle
Stop Solution – 11 mL/bottle of 0.5N HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Antibody Solution SHOULD BE STORED

at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold Concentrate and other components may be stored at 2 - 8°C for up to 6 months. Reconstituted Positive Control should be prepared and used immediately.

Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

SAMPLE DILUTION

Samples may require a 10X-fold dilution. A suggested 10X-fold dilution is 10 µL of sample + 90 µL of dilution buffer. This is only a suggestion. A pretest is needed to determine the optimal dilutions for your samples. Optimal dilutions should be determined by each laboratory for each application. **Use polypropylene test tubes.**

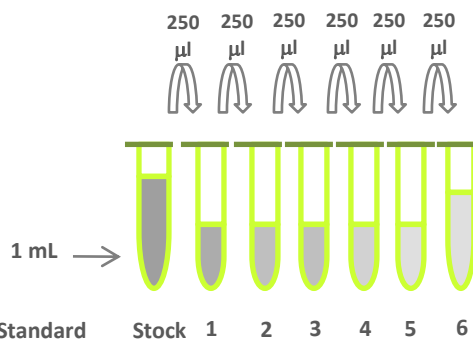
REAGENT PREPARATION

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 Wash Buffer.

NOV/CCN3 Standard - Refer to vial label for reconstitution volume. Reconstitute the **NOV/CCN3 Standard** with 1 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 (see 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	1000 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml



Concentration 2000 1000 500 250 125 62.5 31.25 pg/mL

Positive Control – Reconstitute the **Positive Control** with 1.0 mL Dilution Buffer. *Positive Control should be prepared and used immediately.*

Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution.

Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

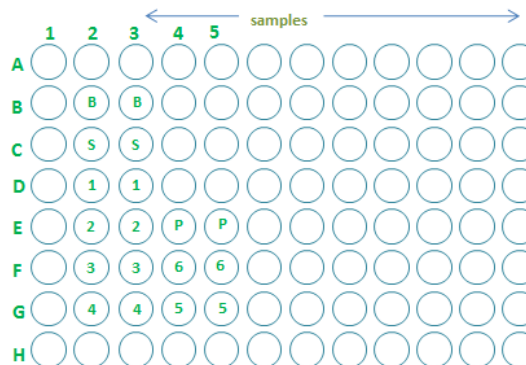
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. *Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (B2, B3).
4. Add 100 µL of **Standard** (from C2, C3 to G2, G3 and F4, F5 to G4, G5), **sample**, or **positive control** (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **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 micro-plate 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 micro-plate 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 3-8 minutes 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 micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. 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 NOV/CCN3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human NOV/CCN3.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of NOV/CCN3 was 15 pg/mL.

TYPICAL DATA

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

NOV/CCN3 (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0.127
31.25	0.038
62.5	0.062
125	0.117
250	0.245
500	0.574
1000	1.237
2000	2.337

*Lot No.:

** Positive Control: 105 – 205 pg/mL

SPECIFICITY

This assay recognizes both natural and recombinant human NOV/CCN3. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

ADIOKINES	CROSSREACTIVITY (%)
HUMAN NOV/CCN3	100
MOUSE NOV	7.7%
HUMAN NOTCH-1/Fc CHIMERA	0
HUMAN S100-A4	0
HUMAN WISP-1	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard, 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 minutes on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 3-8 minutes on the bench top. Protect from light.
↓
Add 100 µL Stop Solution to each well. Read at 450nm within 15 minutes