

HUMAN SOLUBLE NEURAL CELL ADHESION MOLECULE (NCAM-1/CD56) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN sNCAM-1 CONCENTRATIONS
IN CELL CULTURE SUPERNATANTS, SERUM
AND PLASMA



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

PURCHASE INFORMATION:

ELISA Name	Human Soluble NCAM-1 ELISA
Catalog No.	SK00266-01
Lot No.	
Formulation	96 T
Standard Range	62.5 - 4000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µL
Sample Type	Cell Culture Supernatants, Serum and Plasma
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human NCAM-1 only
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8 °C

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INTRODUCTION

Human soluble NCAM-1 immunoassay is a solid phase ELISA designed to measure human NCAM-1 in cell culture supernatants, serum and EDTA plasma. Other sample types, such as CSF, need to be validated prior to assay. This kit contains recombinant human NCAM-1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human NCAM-1. Results obtained with naturally occurring NCAM-1 samples show 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 NCAM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for NCAM-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any NCAM-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for NCAM-1 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 NCAM-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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
NCAM-1 Microplate – 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against NCAM-1.	266-01-01	1 plate
NCAM-1 Standard – 4000 pg/vial of recombinant human NCAM-1 in a buffered protein base with preservative; lyophilized.	266-01-02	1 vial
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against NCAM-1 with preservative; lyophilized.	266-01-03	1 vial
Positive Control – one vial of recombinant human NCAM-1; lyophilized.	266-01-04	1 vial
Streptavidin-HRP Conjugate – 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	DB01	1 bottle
Antibody Diluent Solution Concentrate – 11 mL of buffered protein based solution with preservative; lyophilized.	DB20	1 tube
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
Plastic Pouch	P01	1

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 12 months. For longer storage, unopened Standard, Positive

Control, Detection Antibody Concentrate and Antibody Diluent Solution Concentrate should be stored at -20 °C or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock), Detection Antibody concentrated solution and Antibody Diluent Solution SHOULD BE STORED at -20 °C or -70 °C for up to one month.

Streptavidin-HRP Conjugate 200-fold concentrated solution (**protect from light**) and other components may be stored at 2 – 8 °C for up to 12 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.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

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.

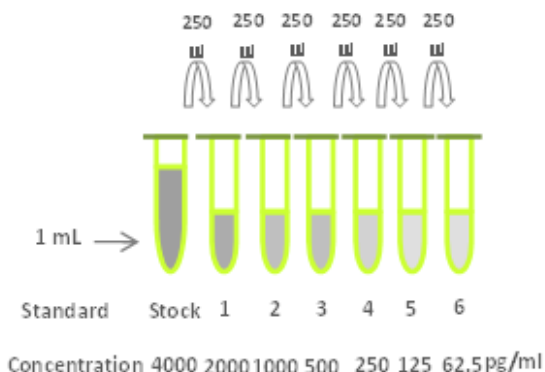
Antibody Diluent Solution Concentrate –

Reconstitute the Antibody Diluent Solution Concentrate with 11.0 mL of Dilution Buffer in provided 15 mL centrifuge tube to prepare Antibody Diluent Solution.

NCAM-1 Standard - Refer to vial label for

reconstitution volume. Reconstitute the NCAM-1 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 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 #1-6. Use the stock standard solution to produce a dilution series. Mix each tube thoroughly before the next transfer. The **4000 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	4000 pg/mL
# 1	250 µl of stock	250 µl	2000 pg/mL
# 2	250 µl of 1	250 µl	1000 pg/mL
# 3	250 µl of 2	250 µl	500 pg/mL
# 4	250 µl of 3	250 µl	250 pg/mL
# 5	250 µl of 4	250 µl	125 pg/mL
# 6	250 µl of 5	250 µl	62.5 pg/mL



Positive Control - Reconstitute the Positive Control tube with 1.0 mL Dilution Buffer. **Note:** Positive Control solution can be reused within a few days if stored at -20 °C to -70 °C.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB20)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody Diluent Solution (DB20) into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. **Note:** Prepare 1-2 hours prior to use.

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 (**protect from light**).

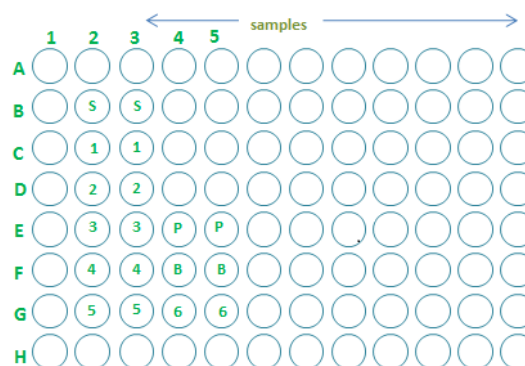
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standard solutions, positive control and samples be assayed in duplicate.

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 of Dilution Buffer to Blank wells (F4, F5).
4. Add 100 µL of Standard solutions in reverse order of serial dilution from #6-S (G4, G5 and G2, G3 to B2, B3), sample, or positive control (E4, E5) per

well. Cover with plate sealer. Incubate for 2 hours on microplate 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 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 2-5 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

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 NCAM-1 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.

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 4000 pg/mL may result in inaccurate, low human NCAM-1 levels. Such samples require further external predilution according to expected human NCAM-1 values with Dilution Buffer in order to precisely quantify the actual human NCAM-1 level.

TYPICAL DATA

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

NCAM-1 (pg/mL)	Average OD450 (Corrected)*
Blank	0 (0.129)
31.25 (optional)	0.019
62.5	0.063
125	0.148
250	0.266
500	0.508
1000	0.968
2000	1.794
4000	2.828

- Lot No.:
- Positive Control:

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human NCAM-1.

SENSITIVITY

The minimum detectable dose (MDD) of NCAM-1 was 15 pg/mL.









SPECIFICITY

This assay recognizes both natural and recombinant human NCAM-1. 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 NCAM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEIN NAME	CROSS-REACTIVITY (%)
Human NCAM-1	100
Human NCAM-L1/Fc Chimera	0
Human BCAM/Fc Chimera	0
Human EpCAM/TROP-1/Fc Chimera	0
Human ALCAM-L1/Fc Chimera	0
Mouse MadCAM-1/Fc Chimera	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. Prepare Detection Antibody Working Solution.

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 2-5 min on the plate shaker at RT. Protect from light.

Add 100 µl Stop Solution to each well. Read 450nm within 15 min.