HUMAN SOLUBLE NEURONAL CELL ADHESION MOLECULE (NRCAM) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN NRCAM CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA.

PURCHASE INFORMATION:

ELISA Name	Human Soluble NrCAM ELISA
Catalog No.	SK00266-01
Formulation	96 T
Standard Range	62-4000 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µl
Sample Type	CSF, Plasma, Serum
Specificity	Human NrCAM
Sample Dilution	*Optimal dilutions should be determined by each laboratory for each application*
Intra-assay Precision	4-6%
Inter-assay Precision	4-8%
Storage	2 °C-8 °C

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

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INTRODUCTION

Human Soluble NrCAM immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human soluble NrCAM in CSF. It contains recombinant human soluble NrCAM and antibodies raised against this protein. It has been shown to accurately quantitie recombinant human NrCAM. Results obtained with naturally occurring NrCAM 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 soluble NrCAM.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for NrCAM has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any NrCAM present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated monoclonal antibody specific for NrCAM is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add 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 NrCAM 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 the appropriate 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.

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.

MATERIALS PROVIDED

Description	Code	Quantity
NrCAM Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal purified IgG against NrCAM.	266-01-01	1 plate
NrCAM Standard – 34 ng/vial of recombinant human NrCAM in a buffered protein base with preservatives; lyophilized.	266-01-02	1 vial
Detection Antibody Concentrate– 120 µL / vial, 100-fold concentrated of Biotinylated monoclonal purified IgG against NrCAM with preservatives; lyophilized.	266-01-03	1 vial
Positive Control- one of recombinant human NrCAM, lyophilized	266-01-04	1 vial
Antibody Dilution Solution-12 ml/vial of buffered protein based solution with preservatives	BD60	1 vial
Streptavidin-HRP Conjugate -75 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial

Dilution Buffer- 45 mL/vial of buffered protein based solution with preservatives	DB01	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution-13 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution（0.5M HCl）, 13 ml /vial of 0.5M HCl	S-STOP	1 vial
Plate Covers – Plate sealer.	EAPS	1

STORAGE

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

Opened / Reconstituted Reagents: *Reconstituted Standard , Antibody Solution and SHOULD BE STORED at -20 °C or – 70°C for up to one months. Reconstituted Positive Control and the diluted Standard solutions CAN NOT BE REUSED.*

Streptavidin - HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. 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.

SAMPLE PREPARATION

CSF samples may require 10-20 fold dilution. A suggested 10 fold dilution is 30 µL of samples + 270 µL Dilution Buffer. A suggested 20 fold dilution is 15 µL of samples + 285 µL Dilution Buffer. 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.

NrCAM Standard - Refer to vial label for reconstitution volume. Reconstitute the **NrCAM** Standard with 1 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 250µL of the appropriate Dilution Buffer into the tube #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

Standard	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 p/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

Detection Antibody- Reconstitute the **Detection Antibody concentrated** with 120 µl of Antibody Dilution Solution to produce a 100-fold concentrated stock solution. Transfer 120 µl of 100-fold concentrated stock solution to 11.88 mL of Antibody Dilution Solution to prepare working solution. This preparation require prior two hours to use.

Streptavidin-HRP Conjugate - Pipette 11. 94 mL of Dilution Buffer into the 15 ml centrifuge tube and transfer 60 µl of 200-fold concentrated stock solution to prepare working solution. *Note: 1 x working solution of Streptavidin-HRP Conjugate should be used within a few days.*

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

ASSAY PROCEDURE

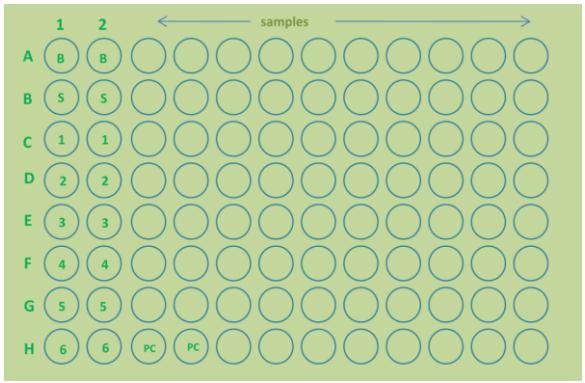
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

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 foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (A1, A2).
4. Add 100 µL of Standard (from B1 to H2), sample, or control per well. Cover with the 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 (300µL) 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 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 40 minutes on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 10-20 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 3 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, 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 four parameter logistic (4-PL) curve fit.

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



TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standard (pg/mL)	Corrected (Average OD450nm)
Blank	0(0.112)
62.5	0.031
125	0.079
250	0.154
500	0.312
1000	0.612
2000	1.126
4000	2.2691

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human NrCAM Extracellular domain.

SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards
↓
Add 100µl 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 to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate to each well. Incubate 40 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate to each well. Incubate 10-20 min on the bench top. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 3 min