SOLUBLE NEPRILYSIN/CD10 (HUMAN) ULTRASENSITIVE ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE NEPRILYSIN/CD10 CONCENTRATIONS IN ANIMAL FREE CELL CULTURE SUPERNATES, SERUM AND PLASMA

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.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**SOLUBLE NEPRILYSIN/CD10 (HUMAN) ULTRASENSITIVE ELISA KIT**
Catalog No.	SK00724-06
Lot No.	
Formulation	96 T
Standard Range	31 - 1000 pg/mL
Sensitivity	10 - 15 pg/mL
Sample Volume	60 µl of sample or diluted sample solution per well
Sample Type	Serum, EDTA Plasma, Animal Free Cell Culture Supernates
Dilution Factor	4~8 (Optimal dilutions should be determined by each laboratory for each application)
Specificity	Human and Rhesus Soluble Neprilysin
Calibration	Human Soluble Neprilysin Fc (HEK293 cell derived)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ORDER CONTACT:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

DESCRIPTION

This Soluble Neprilysin/CD10 (Human) Ultrasensitive ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural soluble Neprilysin from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human soluble Neprilysin Fc and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant human soluble Neprilysin Fc and human soluble Neprilysin derived from HEK293 cells.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with an antibody specific for soluble Neprilysin. The capture antibody can bind to the soluble Neprilysin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against soluble Neprilysin 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, Enhanced Chemifluorescent Substrate working solution is added to the wells for 25 minutes incubation before adding Stop Solution. The ***fluorescent signal was read from top of wells*** using a Fluorescent microplate reader with Excitation at 530-575nm; Emission at 585-630nm.

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.
_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
Neprilysin Microplate - 96 well polystyrene black microplate (12 strips of 8 wells) coated with an antibody against human Neprilysin.	**724-06-01**	**1 plate**
Neprilysin Standard – lot specific of recombinant human soluble Neprilysin in a buffered protein base with preservative; lyophilized.	**724-06-02**	**1 vial**
Detection Antibody Concentrate – lot specific of concentrate of a biotinylated antibody against human soluble Neprilysin with preservative; lyophilized.	**724-06-03**	**1 vial**
Positive Control – one vial of recombinant human soluble Neprilysin; lyophilized.	**724-06-04**	**1 vial**
Streptavidin-HRP Conjugate – 15 µl of 500-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer - 50 mL of buffered protein based solution with preservative.	**DB09**	**1 bottle**
Antibody & HRP Diluent Solution - 20 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffer with preservative.	**WB01**	**1 bottle**
HRP Wash Buffer - 20 mL of 10-fold concentrated buffer with no preservatives.	**WB02**	**1 bottle**
Chemifluorescent Solution A – 3.2 mL of Chemifluorescent substrate solution.	**CFS01A**	**1 bottle**
Chemifluorescent Solution B – 3.2 mL of Chemifluorescent substrate solution.	**CFS01B**	**1 bottle**
Solution C – 65 µL	**CFS-C**	**1 vial**

Stop Solution – 1.0 mL	**CFS-STOP**	**1 vial**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 10 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.
THIS ELISA KIT IS FOR ONE TIME USE ONLY. ANY RECONSTITUTED COMPONENTS CAN NOT BE REUSED.

ADDITIONAL MATERIALS REQUIRED

- Fluorescent microplate reader with excitation/emission at 530~575nm/585~630nm.
- Microplate shaker (350-450 rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

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. Wash immediately with water in case of contact on skin or eyes.

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. ***Note:*** *Bovine serum may cross-react with this ELISA kit, use animal serum free cell culture supernates only.*
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x 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 1000 x 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

Serum or plasma samples may need 4-8 fold dilution. A 4-fold dilution is 40 µL of sample + 120 µL of 1x Dilution Buffer (DB09). A 8-fold dilution is 20 µL of sample + 140 µL of 1x Dilution Buffer (DB09).
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 (WB01) into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

HRP Wash Buffer – Dilute 20 mL of HRP Wash Buffer Concentrate (WB02) into deionized or distilled water (180 mL) to prepare 200 mL of 1x HRP Wash Buffer. ***Note:*** *This wash buffer is for final washing the plate after Step 8 (page 4) and before adding* ***Chemifluorescent Mix Solution*** *only.*

Neprilysin Standard - Reconstitute the human soluble Neprilysin standard with lot specific Dilution Buffer. 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 #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 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	Lot specific	
# 1	Lot specific	Lot specific	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

Positive Control - Reconstitute the Positive Control with lot specific of Dilution Buffer to make working solution. Let it sit for 15 minutes with gentle agitation.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with lot specific of **Antibody & HRP Diluent Solution (DB01)** to produce a 10-fold concentrated stock solution. Pipette 6.3 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 0.7 mL of 10-fold concentrated stock solution to prepare working solution

Streptavidin-HRP Conjugate - Pipette 5.988 mL of **Antibody & HRP Diluent Solution (DB01)** into a 15 mL centrifuge tube and transfer 12 µl of 500-fold concentrated stock solution to prepare working solution (protect from light).

Chemifluorescent Mix Solution – Mix 50 part of Chemifluorescent Solution A (CFS01A) and 50 part of Chemifluorescent Solution B (CFS01B), then add 1 part of Solution C (CFS-C). **Note:** Bring all substrate solutions to room temperature before mixing. DO NOT CONTAMINATE ANY OF THE INDIVIDUAL SOLUTIONS!

For example: To make a 6.0 mL of Chemifluorescent Mix Solution, mix 3.0 mL of Chemifluorescent Solution A (CFS01A) and 3.0 mL of Chemifluorescent Solution B (CFS01B), then add 60 µl of Solution C (CFS-C).

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. Add 60 µL of Dilution Buffer to Blank wells.
3. Add 60 µL of Standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 150 minutes on microplate shaker at room temperature.
4. Aspirate each well and wash with **1x Wash Buffer from WB01**, 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 1x Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 60 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 60 µL of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Wash by hand with **1x HRP Wash Buffer from WB02**, repeating the process three times for a total of four washes. Fill each well with 1x HRP Wash Buffer (300 µL). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1x HRP Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
10. Add 60 µL of Enhanced Chemifluorescent Mix Solution to each well. Incubate for 25-26 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 10 µL of Stop Solution (CFS-STOP) to each well. Shake the plate to ensure thorough mixing.
12. The fluorescent signal was read from top of wells using a fluorescent microplate reader with Excitation at 530-575 nm and Emission at 585-630nm.

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 the reader's software. 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 human soluble Neprilysin concentrations versus the log of the absorbance 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.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Following the fluorescent signal was read from top of wells using a fluorescent microplate reader FilterMaX F5 Multi-Mode Microplate Reader (Molecular Devices) with Excitation at 535 nm and Emission at 625nm.

STANDARD (PG/ML)	RFU
Blank	0 (5.5e7)
31.25	2420246
62.5	3831098
125	7325322
250	12269526
500	21608814
1000	38940446

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Soluble Neprilysin, Fc (HEK293)	100
Human Soluble Neprilysin (HEK293)	100
Rhesus Soluble Neprilysin (HEK293)	100
Human MPF Fc (HEK293)	0
Human sCD146	0
Human sCD163	0
Human Pro BNP	0

The recombinant human soluble Neprilysin derived from *E. Coli* may not be detected by this ELISA Kit. Bovine serum or other animal serum may cross-react with this ELISA kit. Sample dilution can not contain any animal serum or plasma. Please use animal serum free cell culture supernates for cell culture sample assay.

SAMPLE TEST

The pooled research human serum samples were diluted with 1x Dilution Buffer DB09 and assayed by this ELISA Kit.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
4 X	1310.680	5242.721	100
8 X	753.659	6029.273	115
16 X	360.275	5764.408	110

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 60 µl of standard dilutions, samples or positive control to each well. Incubate 150 minutes on the plate shaker at RT.
⬇
Aspirate and wash 4 times with 1x Wash Buffer from WB01.
⬇
Add 60 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times with 1x Wash Buffer from WB01.
⬇
Add 60 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 with 1x Wash Buffer from WB01.
⬇
Aspirate and wash 4 times by hand with 1x HRP Wash Buffer from WB02.
⬇
Add 60 µl Chemifluorescent Mix Solution to each well. Incubate 25-26 minutes on the plate shaker at RT. **Protect from light.**
⬇
Add 10 µl Stop Solution (CFS-STOP) to each well. **Shake the plate before read**. **Read Fluorescent signal from top of wells** with Ex 535nm (530-575nm), Em 625nm (585-630)