HIGH SENSITIVITY HUMAN SOLUBLE NEPRILYSIN/CD10 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE NEPRILYSIN/CD10 CONCENTRATIONS IN SERUM AND EDTA PLASMA

THIS PROTOCOL AND DATA IS PROVIDED FOR DEMONSTRATION ONLY.
ALWAYS REFER TO LOT SPECIFIC PROTOCOL 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	**HIGH SENSITIVITY HUMAN SOLUBLE NEPRILYSIN/CD10 ELISA**
Catalog No.	SK00724-09
Lot No.	
Formulation	96 T
Standard range	15.6-1000 pg/mL
Sensitivity	3 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Human Soluble Neprilysin
Calibration	Human Soluble Neprilysin-Fc (HEK293)
Intra-assay Precision	2 - 4%
Inter-assay Precision	4 - 8%
Storage	2 – 8° C for 8 months. For longer storage check more information on page 2
This kit contains sufficient materials to run approximately 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
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.net

DESCRIPTION

This High Sensitivity Human Soluble Neprilysin/CD10 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural soluble human Neprilysin from serum and plasma in a sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Neprilysin. The capture antibody can bind to the human Neprilysin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human 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, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human Neprilysin 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.

_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 microplate (12 strips of 8 wells)	**724-09-01**	**1 plate**
Neprilysin Standard – 4ng of recombinant human Neprilysin-Fc lyophilized.	**724-09-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL of biotinylated antibody; lyophilized.	**724-09-03**	**1 vial**
Positive Control - one vial ; lyophilized.	**724-09-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB11C**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer – 25 mL of 20-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 0.25M 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 long-term storage up to 12 months, place unopened Standard, Positive Control, and Detection Antibody Concentrate in a freezer at -20° C or -70° C. For Longer storage for Dilution Buffer (**DB10**), Antibody Diluent Solution (**DB11C**) and HRP Diluent Solution (**DB08B**) store in a freezer at -20° C. Store **Streptavidin-HRP Conjugate in** 2 – 8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (200-300 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

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.

SAMPLE PREPARATION

Serum or EDTA plasma samples may need to be diluted by 2 ~8 fold. For 2-fold dilution, add 50 µl of sample per well 50 µl of Dilution Buffer per well. For 4-fold dilution, add 25 µl of sample per well 75 µl of Dilution Buffer per well. For 8-fold dilution, add 12.5 µl of sample per well + 87.5 µl of Dilution Buffer per well.

Optimal dilutions; however, should be determined by each laboratory for each application.
Use polypropylene test tubes.

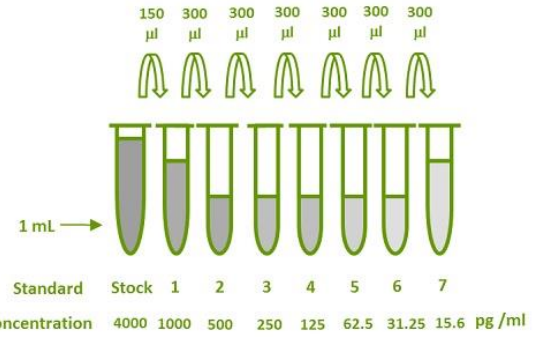
REAGENT PREPARATION

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 25 mL of Wash Buffer Concentrate into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

Neprilysin Standard - Reconstitute the Neprilysin standard with 1mL of **Dilution Buffer (DB10)**. Pipette 300 µL of Dilution Buffer (DB10) into tubes #2 to #7. 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 (DB10) serves as the zero standard (0 pg/mL). Store the stock solution at -70 C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1 mL	4000 pg/ml
# 1	150 µl of stock	450 µl	1000 pg/ml
# 2	300 µl of 1	300 µl	500 pg/ml
# 3	300 µl of 2	300 µl	250 pg/ml
# 4	300 µl of 3	300 µl	125 pg/ml
# 5	300 µl of 4	300 µl	62.5 pg/ml
# 6	300 µl of 5	300 µl	31.25 pg/ml
# 7	300 µl of 6	300 µl	15.6 pg/ml



Positive Control - Reconstitute the Positive Control with 1ml of **Dilution Buffer (DB10)**. Discard the positive control after use.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB11C)** to produce a 10-fold concentrated stock solution. Freshly Pipette 9.45 mL of Antibody Diluent Solution (**DB11C**) into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. The working solution should be used in 10 minutes. For the partial strip test, freshly prepare 900 µl per strip of working solution. Store the stock

solution of 10-fold concentrated antibody solution at -20 °C for a few days.

Streptavidin-HRP Conjugate – Freshly Pipette 11.88 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Protect from light. The 1 x working solution should be used in 10 minutes.** For the partial strip test, freshly prepare 900 µl per strip of working solution. Store the stock solution of 10-fold concentrated antibody solution at -20 °C for a few days.

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 100 µL of **Dilution Buffer (DB10)** to Blank wells.
3. Add 100 µL of **Standard dilutions** in reverse order of serial dilution, **samples**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker (200-300 rpm) at room temperature.
4. 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.
5. 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.
6. Repeat the aspiration/wash as in step 4.
7. 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.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for 20 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 ~ 5 minutes.

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

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Soluble Neprilysin (HEK293)	100
Human Soluble Neprilysin Fc (HEK293)	100
Mouse Neprilysin (HEK293)	0
Human ECE-1 (HEK293)	0
Human ECE-2 (HEK293)	0
Human ACE2-Fc (HEK293)	0

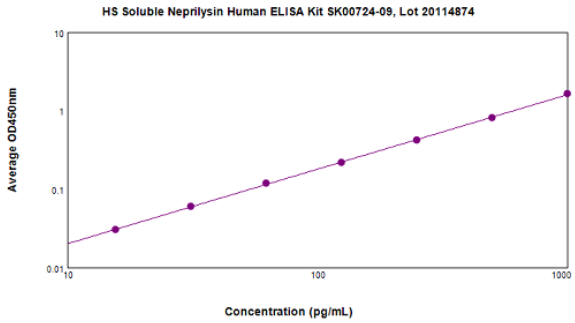
TYPICAL DATA

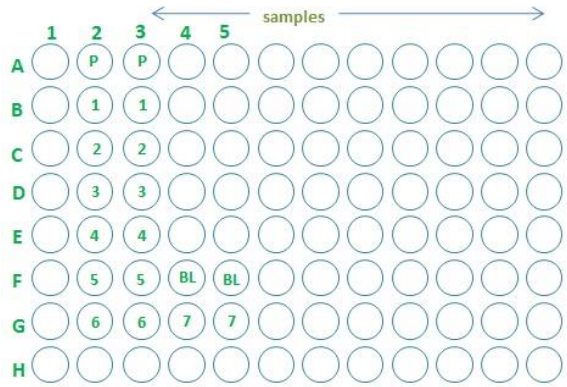
This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.089)
15.6	0.030
31.25	0.060
62.5	0.118
125	0.216
250	0.420
500	0.809
1000	1.616

LOT NO.:20114874

STANDARD CURVE FIT BY LOG-LOG





SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker (200-300 rpm) 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 20 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.

Research Samples Test:

The research samples were diluted by Dilution Buffer DB10. Its linearity and recovery was assayed by HS s NEP (H) ELISA Kit SK00724-09

Sample	Dilution Factors	Assayed (pg/ml)	Final (pg/ml)	Recovery (%)
Human Serum	2 X	467.927	935.854	100
Human Serum	4 X	244.221	929.115	99
Human EDTA Plasma	8 X	117.466	939.727	100
Human EDTA Plasma	2 X	493.084	986.168	100
Human EDTA Plasma	4 X	262.880	1051.52	107
Human EDTA Plasma	8 X	127.073	1016.584	103