

HIGH-SENSITIVITY SOLUBLE NEPRILYSIN (NEP) (HUMAN) ELISA KIT

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



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 SOLUBLE NEPRILYSIN (NEP) (HUMAN) ELISA
Catalog No.	SK00724-01
Lot No.	
Formulation	96 T
Standard range	15.6-1000 pg/mL
Sensitivity	15.6 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 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C for 1 month. More information check page 2-3
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This High-Sensitivity Soluble Neprilysin (NEP) (Human) 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) coated with an antibody against Neprilysin.	724-01-01	1 plate
Neprilysin Standard – refer to lot of recombinant human Neprilysin in a buffered protein base with preservative; lyophilized.	724-01-02	1 vial
Detection Antibody Concentrate – refer to lot, 10-fold concentrate of biotinylated antibody against Neprilysin with preservative; lyophilized.	724-01-03	1 vial
Positive Control - one vial of recombinant human Neprilysin; lyophilized.	724-01-04	1 vial
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	DB03	1 bottle
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB108A	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB08C	1 bottle
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB02	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 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For long-term storage up to 10 months, place

unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 – 8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (400-450 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 4 ~ 16. **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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Dilution Buffer (DB03) - Dilution Buffer (DB03) is highly viscous, warm in 30 - 37° C water bath until liquid flows more freely.

Neprilysin Standard - Reconstitute the Neprilysin standard with refer to lot of **Dilution Buffer (DB03)**. 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 (DB03) into tubes #1 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 (DB03) serves as the zero standard (0 pg/mL). Optional set 2000pg/mL as the highest standard.

TUBE	STANDARD	DILUTION BUFFER (DB09)	CONCENTRATION
stock	Powder	Refer to lot	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
# 7	250 µl of 6	250 µl	15.6 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot of **Dilution Buffer (DB03)**.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with refer to lot of **Antibody Diluent Solution (DB108A)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB108A)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 10.89 mL of **HRP Diluent Solution (DB08C)** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution. **Protect from light.** The working solution of Streptavidin-HRP should be used in a few hours.

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 (DB03)** 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 **3 hours** on microplate shaker (400-450 rpm) at room temperature. Optional incubating for 14 hours at 2-8 °C.
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 refer to lot 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. 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.

11. Determine the optical density of each well using a microplate reader set to 450 nm within 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 or 4-parameter 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 Neprilysin 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.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Soluble Neprilysin	100
Mouse Neprilysin	25.9
Human ECE-1	0
Human ECE-2	0
Human Neprilysin-2	0
Mouse Kell	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 (refer to lot)
15.6	0.037
31.25	0.071
62.5	0.139
125	0.247
250	0.469
500	0.912
1000	1.619
2000	2.798

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 3 hours on the plate shaker (400-450 rpm) at RT. Optional incubating for 14 hours at 2-8 °C.

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 refer to lot on the plate shaker at RT. Protect from light.

Add 100 µl Stop Solution to each well. Read at 450nm within 5 minutes.