SOLUBLE NEPRILYSIN (HUMAN) ULTRASENSITIVE ELISA KIT

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

PURCHASE INFORMATION:

ELISA NAME	SOLUBLE NEPRILYSIN ULTRASENSITIVE ELISA KIT
Catalog No.	SK00724-05
Lot No.	
Formulation	96 T
Standard Range	31 - 2000 pg/mL
Sensitivity	17 pg/mL
Sample Volume	60 µl
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human , Rhesus Soluble Neprilysin
Calibration	Human Soluble Neprilysin Fc (HEK293 cell derived)
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2 - 8° C

THIS ELISA KIT IS ONE TIME USE ONLY.

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INTRODUCTION

Soluble Neprilysin Ultrasensitive immunoassay Kit is a solid phase ELISA designed to measure human soluble neprilysin in animal free cell culture supernates, serum, and plasma. It contains recombinant human soluble Neprilysin Fc and antibodies raised against this protein. It has been shown to accurately quantify recombinant human soluble Neprilysin Fc, human soluble Neprilysin from HEk293 cells. Results obtained with naturally occurring the serial dilution of soluble Neprilysin in human serum 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 Neprilysin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for soluble Neprilysin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Soluble Neprilysin present is bound by the immobilized antibody. After washing away any unbound substances, abiotinylated antibody specific for human soluble neprilysin is added to the wells. Following a wash to remove any unbound antibody reagent, Streptavidin HRP is added to the wells. After a final wash cycle, Chemifluorescent Substrate working solution was added for 28-35 minutes incubation before adding the Stop Solution. The fluorescent signal was measured using a Fluorescent microplate reader with excitation/emission at 325/420nm.

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 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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Neprilysin Microplate - 96 well polystyrene black microplate (12 strips of 8 wells) coated with an antibody against human soluble Neprilysin.	**724-05-01**	**1 plate**
Neprilysin Standard – refer to package label of recombinant human soluble Neprilysin Fc in a buffered protein base with preservative; lyophilized.	**724-05-02**	**1 vial**
Detection Antibody Concentrate – refer to package label of vial, 100-fold concentrated of a biotinylated antibody against soluble Neprilysin with preservative; lyophilized.	**724-05-03**	**1 vial**
Positive Control – one vial of recombinant human soluble neprilysin Fc; lyophilized.	**724-05-04**	**1 vial**
Streptavidin-HRP Conjugate – refer to package label of vial, 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer- 60 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**
Chemifluorescent Solution A – 8 mL of Chemifluorescent substrate solution.	**CFS02A**	**1 bottle**
Chemifluorescent Solution B – 1 mL of Chemifluorescent substrate solution.	**CFS02B**	**1 bottle**
STOP Solution C – 7 mL/vial	**CFS02-STOP**	**1 vial**
Plate Sealer	**EAPS**	**1**

Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 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 IS ONE TIME USE ONLY.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 month at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Fluorescent microplate reader with excitation/emission at 325/420nm.
- 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. Due the bovine serum may interact with this elisa kit, please use animal serum free cell culture medium 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

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 1x Wash Buffer.

Neprilysin Standard - Refer to vial label for reconstitution volume. Reconstitute the soluble human neprilysin standard with refer to lot specific package label of Dilution Buffer. This reconstitution produces a stock solution of refer to lot specific package label 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 #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	Refer to lot specific	
# 1	Refer to lot specific protocol		2000 pg/ml
# 2	250 µl of 1	250 µl	1000 pg/ml
# 3	250 µl of 2	250 µl	500 pg/ml
# 4	250µl 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
# 7	250 µl of 6	250 µl	31.25 pg/ml

Positive Control - Reconstitute the Positive Control stock with 1 mL of Dilution Buffer. Allow the standard to sit for a minimum of 15 minutes with gentle agitation. Positive control should be prepared and used immediately.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to package label of Dilution Buffer to produce a 10-fold concentrated stock solution.

Streptavidin HRP Conjugate - Pipette 6.93 mL of **Dilution Buffer** into a 15 ml centrifuge tube and transfer 70 µl of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin HRP conjugate should be used immediately.

Chemifluorescent Mix Solution – Mix 7.2 ml (9 parts) of Solution A and 0.8 mL (1 part) of Solution B. **Note:** Bring all substrate solutions to room temperature before use. This Chemifluorescent Mix Solution should be used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, 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 60 µL of Dilution Buffer to Blank wells.
4. Add 60 µL of Standard solutions in reverse order of serial dilution, sample, or positive control 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 with **1x Wash Buffer**, repeating the process three times for a total of four washes. Wash by filling each well with 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 60 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 60 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 60 µL of Chemifluorescent Mix Solution to each well. Incubate for 30 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 60 µL of Stop Solution C to each well. Shake the plate to ensure thorough mixing.
12. The fluorescent signal was measured using a fluorescent microplate reader with excitation/emission at 325/420nm.

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 standard 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.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human soluble Neprilysin Fc derived from HEK293 cells.

SENSITIVITY

The minimum detectable dose (MDD) of human soluble Neprilysin was 17 pg/mL.

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)	RFU
31.25	165540
62.5	299911
125	565412
250	1092701
500	2042183
1000	3972458
2000	7126725

SPECIFICITY

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

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 60 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times with 1x Wash Buffer.
↓
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.
↓
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.
↓
Add 60 µl Chemifluorescent Mix Solution to each well. Incubate 30 min on the plate shaker at RT. **Protect from light.**
↓
Add 60 µl Stop Solution to each well. Read Fluorescent signal with Ex 325nm, Em 420nm