# MOUSE NEPRILYSIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE NEPRILYSIN CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA

## PURCHASE INFORMATION:

| ELISA NAME | MOUSE NEPRILYSIN ELISA |
|---|---|
| Catalog No. | SK00724-03 |
| Lot No. | |
| Formulation | 96 T |
| Standard range | 93 - 6000 pg/mL |
| Sensitivity | 10 pg/mL |
| Sample Volume | 100 µL |
| Sample Type | Cell Culture Supernates, Serum and Plasma |
| Dilution Factor | 2 (Optimal dilutions should be determined by each laboratory for each application) |
| Specificity | Mouse Neprilysin |
| Calibration | Mouse Neprilysin recombinant |
| Intra-assay Precision | 4-6% |
| Inter-assay Precision | 8-12% |
| Storage | 2 - 8° C |

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.

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## DESCRIPTION

This Mouse Neprilysin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural mouse Neprilysin from cell culture supernates, serum and plasma in a sandwich ELISA format.

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

## ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for mouse Neprilysin. The capture antibody can bind to the mouse Neprilysin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against mouse 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 mouse 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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. | **724-03-01** | **1 plate** |
| **Neprilysin Standard –** 6000 pg/vial of recombinant mouse Neprilysin in a buffered protein base with preservative; lyophilized. | **724-03-02** | **1 vial** |
| **Detection Antibody Concentrate –** 1.05 mL/vial of 10-fold concentrate of biotinylated antibody against Neprilysin with preservative; lyophilized. | **724-03-03** | **1 vial** |
| **Positive Control -** one vial of recombinant mouse Neprilysin; lyophilized. | **724-03-04** | **1 vial** |
| **Streptavidin-HRP Conjugate –** 60 µL/vial of 200-fold concentrated solution of Streptavidin conjugate to HRP. | **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 buffered surfactant, with preservative. | **WB01** | **1 bottle** |
| **TMB Substrate Solution -** 11 mL of TMB substrate solution. | **TMB01** | **1 bottle** |
| **Stop Solution** – 11 mL of 0.5M HCl. | **S-STOP** | **1 bottle** |
| **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.
**Opened / Reconstituted Reagents:** Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution (protect

from light) and other components may be stored at 2 – 8 °C for up to 6 months.
**Microplate Wells:** Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C.

## ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 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

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
**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 EDTA plasma samples may need a 2-fold dilution. A suggested 2-fold dilution is 125 µL sample + 125 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application with a pretest.**
**Use polypropylene test tubes.**

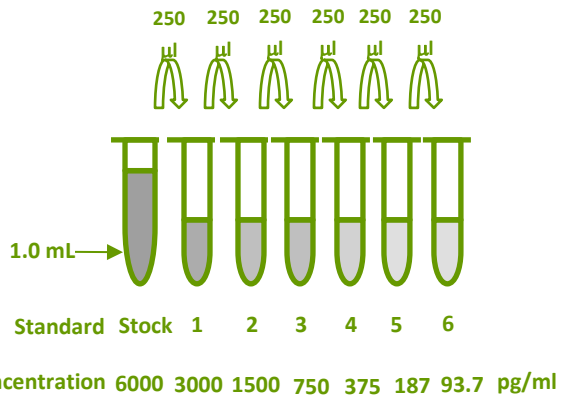
## REAGENT PREPARATION

**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 -** Reconstitute the Neprilysin Standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 6000 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 #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **6000 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 | 1000 µl | 6000 pg/ml |
| **# 1** | 250 µl of stock | 250 µl | 3000 pg/ml |
| **# 2** | 250 µl of 1 | 250 µl | 1500 pg/ml |
| **# 3** | 250 µl of 2 | 250 µl | 750 pg/ml |
| **# 4** | 250 µl of 3 | 250 µl | 375 pg/ml |
| **# 5** | 250 µl of 4 | 250 µl | 187.5 pg/ml |
| **# 6** | 250 µl of 5 | 250 µl | 93.75 pg/ml |




**Positive Control** - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive

Control could be reused within a few days if stored at -20 °C or -70 °C.

**Detection Antibody Concentrate -** Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer 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 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).

## 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. Remove excess microplate strips from the plate frame and put them into the plastic pouch with the desiccant pack.
3. Add 100 µL of **Dilution Buffer** to Blank wells.
4. Add 100 µL of **Standard dilutions** 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.
5. 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.
6. 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.
7. Repeat the aspiration/wash as in step 5.
8. 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.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 5-10 minutes on microplate shaker 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 15 minutes, using a microplate reader set to 450 nm.

## CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

## SPECIFICITY

| PROTEINS | CROSS-REACTIVITY (%) |
|---|---|
| Mouse Neprilysin | 100 |
| Human Neprilysin | 9 |
| Mouse Kell | 0 |
| Human NEP-2/MMEL-2 | 0 |
| Human ECE-1 | 0 |
| Human ECE-2 | 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 (0.080) |
| **46.875 (optional)** | 0.009 |
| **93.75** | 0.025 |
| **187.5** | 0.051 |
| **375** | 0.111 |
| **750** | 0.204 |
| **1500** | 0.401 |
| **3000** | 0.708 |
| **6000** | 1.221 |

- Lot:
- Positive control:

## LINEARITY

To assess the linearity of the assay pooled research mouse serum samples were diluted with **Dilution Buffer (DB01)** and assayed.

| DILUTION FACTOR | ASSAYED (PG/ML) | FINAL (PG/ML) | RECOVERY (%) |
|---|---|---|---|
| **2x** | 721.298 | 1442.596 | 100 |
| **4x** | 338.300 | 1353.2 | 93.8 |

To assess the linearity of the assay pooled research mouse plasma samples were diluted with **Dilution Buffer (DB01)** and assayed.

| DILUTION FACTOR | ASSAYED (PG/ML) | FINAL (PG/ML) | RECOVERY (%) |
|---|---|---|---|
| **2x** | 351.485 | 702.97 | 100 |
| **4x** | 174.563 | 698.252 | 99.3 |

## SUMMARY OF ASSAY PROCEDURE

| PREPARE REAGENTS, SAMPLES AND STANDARDS |
|---|
| ↓ |
| Add 100 µl of standard dilutions, samples, positive control to the well. Incubate 2 hours on the plate shaker 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 5-10 min on the plate shaker at RT. **Protect from light**. |
| ↓ |
| Add 100 µl Stop Solution to each well. Read 450nm within 15 min. |