HUMAN SOLUBLE NEUROPILIN 1 (NRP1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE NRP1 CONCENTRATIONS IN SERUM AND EDTA 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	HUMAN SOLUBLE NEUROPILIN 1 ELISA KIT
Catalog No.	SK00270-01
Formulation	96 T
Lot No.	20113935
Standard range	0.3125 -20 ng/mL
Sensitivity	100 pg/mL
Sample Volume	100 µL
Dilution Factor	40 ~80 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma
Specificity	Human Soluble NRP1
Calibration	Human Soluble NRP1 HEK293 derived
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C for 1 month. See page 2-3 for detail
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble Neuropilin 1 (NRP1) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human NRP1 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human soluble NRP1 derived from HEk293 and monoclonal antibody and antigen affinity purified antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural NRP1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human NRP1. The capture antibody can bind to the human NRP1 in the standard and samples. After washing the plate of any unbound substances, the biotinylated antibody against human NRP1 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 NRP1 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.
_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
NRP1 Microplate – 96 well microplate coated with antibody specific for human NRP1.	**270-01-01**	**1 plate**
NRP1 Standard – 20 ng per vial of lyophilized recombinant human NRP1 (HEK293).	**270-01-02**	**1 vial**
Detection Antibody Concentrate – 0.6 mL of 10-fold concentrate of lyophilized biotinylated antibody against human NRP1.	**270-01-03**	**2 vials**
Positive Control– one vial of lyophilized recombinant human NRP1.	**270-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered solution with preservative.	**DB03**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered solution with preservative.	**DB12**	**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 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and 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 (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

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store 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) (Aviscera Bioscience's Catalog : 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

Due soluble NRP1 in milk has high concentration, please do not use any buffer solution contains dried milk to dilute sample solution or detection antibody.

SAMPLE PREPARATION

Serum or EDTA Plasma samples may require at least a 40-fold ~ 80-fold dilution. A suggested 40-fold dilution is 10 µl sample + 390 µl Dilution Buffer. A suggested 80-fold dilution is 125 µl of 40-fold diluted sample + 125 µl Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.

Use polypropylene test tubes.

REAGENT PREPARATION

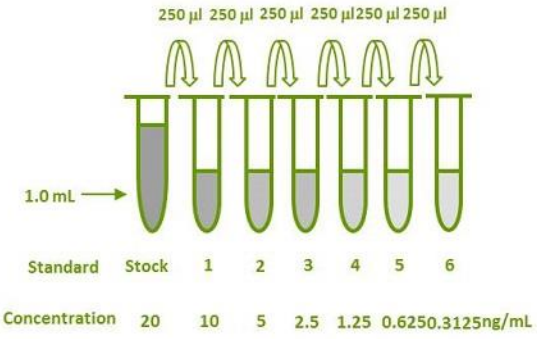
Bring all reagents to room temperature before use.

Wash Buffer – If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 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.**

NRP1 Standard – Reconstitute the NRP1 standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 20ng/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. Create a standard curve using a 2-fold serial dilution in Dilution Buffer with a high standard of **20 ng/mL** is recommended.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	1mL	20 ng/mL
# 1	250 µL of 1	250 µL	10 ng/mL
# 2	250 µL of 1	250 µL	5 ng/mL
# 3	250 µL of 2	250 µL	2.5 ng/mL
# 4	250 µL of 3	250 µL	1.25 ng/mL
# 5	250 µL of 4	250 µL	0.625 ng/mL
# 6	250 µL of 5	250 µL	0.3125 ng/mL



Positive Control - Reconstitute the Positive Control with 1ml of Dilution Buffer to prepare working solution.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 0.6 mL of **Dilution Buffer (DB03)** to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Pipette 5.4 mL of **Dilution Buffer (DB03)** into a 15 mL centrifuge tube and transfer 0.6 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin HRP Conjugate - Pipette 9.395 mL of **HRP Diluent Solution (DB12)** into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light)**. **DO NOT FREEZE.** *The working solution of Streptavidin-HRP Conjugate should be freshly prepared.*

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, standard dilutions, positive control and samples as directed previously.
2. Add 100 µL per well of **Dilution Buffer** to Blank (B) wells. Please refer to well position in page 5.
3. Add 100 µL per well of **Standard dilutions (7 to 1)**, **samples,** or **positive control (P)**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
5. Add 100 µL per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration and wash as in step 4.
7. Add 100 µL per well of **Streptavidin HRP Conjugate working solution**. Cover with plate sealer and incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration and wash as in step 4.
9. Add 100 µL per well of **Substrate Solution**. Incubate for 14-18 min on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Read plate using a microplate reader set to 450 nm within 3 minutes.

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 or 4-parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted by 40-fold or 80-fold, the concentration read from the standard curve must be multiplied by the dilution factor 40 or 80.

SPECIFICITY

Protein	Cross-reactivity (%)
Human Soluble NRP1	100
Human Soluble VEGF-R1	0
Human Soluble VEGF-R2	0
Human VEGF-R3	0

TYPICAL STANDARD CURVE

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.189)
312.5	0.042
625	0.109
1250	0.217
2500	0.397
5000	0.793
10000	1.509
20000	2.214

Lot No.: 20113935
Positive Control: 1500 ~ 7000 pg/mL

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
↓
Add 100 µL of standard dilutions, samples or positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Streptavidin HRP Conjugate working solution. Cover with plate sealer and incubate 60 minutes on microplate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate 14-18 min on microplate shaker at RT. **Protect from light.**
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 3 min.