HUMAN SOLUBLE NEUREGULIN 1-β1 (NRG 1-β1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN NRG 1-β1 CONCENTRATIONS IN SERUM AND EDTA PLASMA

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA Name	Human NRG 1-β1 ELISA
Catalog No.	SK00556-01
Lot No.:	
Formulation	96 T
Standard Range	62.5 - 4000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma
Specificity	Human NRG 1-β1
Sample Dilution	8 *(Optimal dilutions should be determined by each laboratory for each application.)*
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2-8°C

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INTRODUCTION

Human NRG 1-β1 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human NRG 1-β1 in serum and EDTA plasma. It contains recombinant human NRG 1-β1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human NRG 1-β1. Results obtained with naturally occurring NRG 1-β1 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 NRG 1-β1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for NRG 1-β1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any NRG 1-β1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for NRG 1-β1 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of NRG 1-β1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.

_Some vials contain small quantities of material, therefore centrifuge before use.

MATERIALS PROVIDED

Description	Code	Quantity
NRG 1-β1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against NRG 1-β1.	**556-01-01**	**1 plate**
NRG 1-β1 Standard – 4000 pg/vial of recombinant human NRG 1-β1 in a buffered protein base with preservatives; lyophilized.	**556-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against NRG 1-β1 with preservatives; lyophilized.	**556-01-03**	**1 vial**
Positive Control – one vial of recombinant human NRG 1-β1, lyophilized	**556-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB08**	**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) and Detection Antibody Concentrated Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-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 and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- 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

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

Because sNRG1-beta1 levels in serum or plasma samples vary widely, ***optimal dilutions should be determined by each laboratory for each application with a pretest.***

It is recommended to directly assay all samples first. If samples levels are less than 250 pg/mL lyophilize 500 µL of samples and reconstitute it with 250 µL of Dilution Buffer, this will concentrate that sample by 2 times. If sample levels are higher than 4000 pg/mL, it may require an 8-fold or higher dilution. A suggested 8-fold dilution is 30 µL sample + 210 µL Dilution Buffer.
Use polypropylene test tubes.

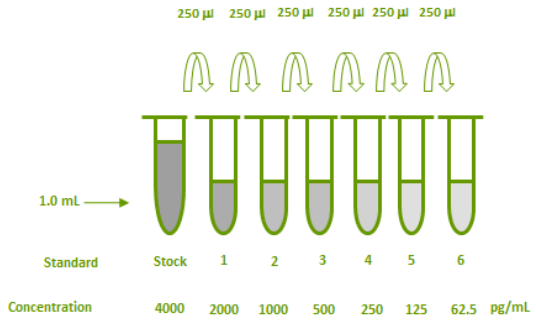
PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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 Wash Buffer.
NRG 1-β1 Standard - Refer to vial label for reconstitution volume. Reconstitute the **NRG 1-β1** standard with 1.0mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of the appropriate 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 4000 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	1000 µl	4000 pg/ml
# 1	250 µl of stock	250 µl	2000 pg/ml
# 2	250 µl of 1	250 µl	1000 pg/ml
# 3	250 µl of 2	250 µl	500 pg/ml
# 4	250 µl 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



Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control solution. ***Note:*** *Positive Control should be used within a few days if stored at -20°C ~ -70°C.*

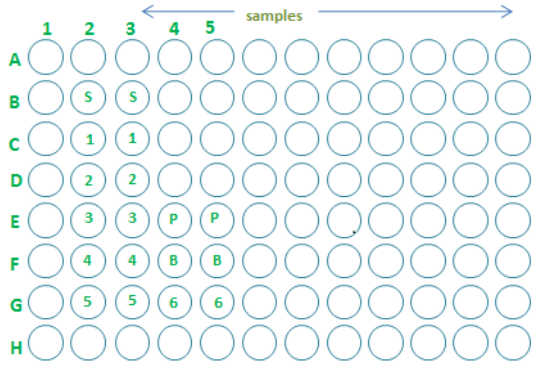
Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer 1.05 mL of 10-fold concentrated stock solution to 9.45 mL of Dilution Buffer to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-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).*

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 micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of Dilution Buffer to Blank wells (F4, F5).
4. Add 100 µL of Standard (from B2, B3 to G2, G3 and G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
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 micro-plate 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 1 hour on micro-plate 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 12-18 minutes on micro-plate 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 micro-plate reader set to 450 nm.



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. 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 NRG

1-β1 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.

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)	CORRECTED OD450nm
Blank	0 (0.170)
62.5	0.025
125	0.028
250	0.058
500	0.115
1000	0.295
2000	0.645
4000	1.148

- Lot No.:
- Positive Control:

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human NRG 1-β1.

SENSITIVITY

15 pg/mL

SPECIFICITY

This assay recognizes both natural and recombinant human NRG 1-β1. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rh NRG 1-β1 control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY (%)
Human NRG 1-β1	100
Human ErbB2/Fc chimera	0
Human ErbB3/Fc chimera	0
Human NRG4 extracellular domain	0
Human NRG 1 –α (EGF domain)	0
Human NRG1 isoform SMDF	0

LINEARITY

To assess the linearity of the assay, pooled research human **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (pg/mL)	FINAL (pg/mL)	RECOVERY (%)
4x	7318.906	29275.624	100
8x	3467.657	27741.256	94.8
16x	1645.224	26323.584	89.9

To assess the linearity of the assay, pooled research human **EDTA plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (pg/mL)	FINAL (pg/mL)	RECOVERY (%)
4x	6001.472	24005.888	100
8x	3078.534	24628.272	103
16x	1404.607	22473.712	93.6

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each 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 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 12-18 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min