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Neuregulin-1/NRG-1-beta1 (human) ELISA Kit

(Catalog # K4711-100, 100 assays; Store at -20^{0}C)

I. Introduction:

Neuregulin-1 (NRG-1) belongs to the neuregulin family of proteins which act via the EGFR receptor signaling pathway. NRG-1 is a glycoprotein with many splice variants. It plays a critical role in the growth and development of multiple organ systems like the nervous system and the heart. Dysregulation of the NRG-1 gene has been linked to diseases such as cancer, schizophrenia, and bipolar disorder. BioVision's human NRG-1 ELISA Kit is based on the standard principle of a sandwich enzyme-linked immunosorbent assay. A mouse monoclonal antibody specific for human NRG-1 is coated on a 96-well plate. Standards and test samples are added to the wells and NRG-1 present in a sample is bound by the immobilized antibody. A biotinylated polyclonal detection antibody from goat specific for NRG-1 is added subsequently. After washing away the unbound biotinylated antibody with PBS or TBS buffer, HRP conjugate is added to the wells. The wells are again washed with PBS or TBS buffer to remove the unbound conjugates. HRP substrate TMB is used to visualize the HRP enzymatic reaction. TMB is used by HRP to produce a blue product that changes into yellow after adding acidic stop solution. The density of yellow color is proportional to the human NRG-1 captured onto the plate. This ELISA kit shows no detectable cross-reactivity with related proteins. Detection Range: 62.5 pg/ml – 4000 pg/ml. Sensitivity: < 10 pg/ml. Coefficient of variation: 5% (Intra-assay) and 7% (Inter-assay).

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Native and recombinant human NRG-1

IV. Sample Type:

- Serum & plasma (heparin, EDTA)
- Cell culture supernatants

V. Kit Contents:

Components	K4711-100	Part No.
NRG-1 Ab coated plate	12 strips x 8 wells	K4711-100-1
Human NRG-1 Standard	2 vials (10 ng/vial)	K4711-100-2
Detection antibody (100x)	130 µl	K4711-100-3
HRP Conjugate (100x)	130 µl	K4711-100-4
Sample diluent buffer	30 ml	K4711-100-5
Antibody diluent buffer	12 ml	K4711-100-6
HRP diluent buffer	12 ml	K4711-100-7
TMB Substrate	10 ml	K4711-100-8
Stop Solution	10 ml	K4711-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended for large number of samples.
- Washing buffer (neutral PBS or TBS).
  - Preparation of 0.01 M TBS: Add 1.2 g Tris, 8.5 g NaCl; 450 µl of purified acetic acid or 700 µl of concentrated hydrochloric acid to 1000 ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
  - Preparation of 0.01 M PBS: Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

VII. Storage Conditions and Reagent Preparation:

Store kit at 4^{o}C for 6 months or at -20^{o}C for 12 months. Avoid repeated freeze-thaw cycles. Centrifuge tubes briefly to spin down all components to the bottom before opening.

- **Reconstitution of the human NRG-1 Standard**: Two vials of NRG-1 standard (10 ng per vial) are included in each kit. Use one vial for each experiment. Prepare 10 ng/ml of human NRG-1 standard solution by adding 1 ml of sample diluent buffer into one of the vials. Keep the tube at room temperature for 10 min. and mix thoroughly. Add 0.4 ml of the 10 ng/ml solution into 0.6 ml sample diluent buffer and mix thoroughly to make 4000 pg/ml stock. Label 6 Eppendorf tubes with 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the 4000 pg/ml NRG-1 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.
  Note: The standard solutions are best used within 2 hrs. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hrs, or at -20°C for up to 48 hrs. Avoid repeated freeze-thaw cycles.
- **Preparation of Detection antibody working solution**: Dilute 1:100 with the antibody diluent buffer and mix thoroughly. Prepare 0.1 ml of antibody working solution for each well. Solution should be prepared no more than 2 hrs. prior to the experiment.
- **Preparation of HRP conjugate working solution**: Dilute 1:100 with the HRP diluent buffer and mix thoroughly. Prepare 0.1 ml of ABC working solution for each well. Solution should be prepared no more than 1 hr. prior to the experiment.

VIII. Sample Preparation and Storage:

Centrifuge cell culture supernatants to remove particulates, assay immediately or aliquot and store at -20°C. Allow the serum to clot in a serum separator tube (about 4 hrs) at room temperature. Centrifuge at approximately 1000 x g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C. Collect plasma using anticoagulants such as heparin or EDTA. Centrifuge for 15 min. at 1500 x g within 30 min. of collection. Analyze immediately or aliquot and store frozen at -20°C.

Notes:

a. Store samples to be assayed within 24 hrs. at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

b. Sample dilution guidelines: The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime of the standard curve. Dilute the sample using the provided diluent buffer. The sample must be well mixed with the sample diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary to optimize sample dilution. For high target protein concentration (40-400 ng/ml): dilute 1:100. For medium target protein concentration (4-40 ng/ml): dilute 1:10. For low target protein concentration (62.5-4000 pg/ml): dilute 1:2. For very low target protein concentration (≤62.5 pg/ml). No dilution necessary or dilute 1:2.

IX. Assay Protocol:

The HRP conjugate working solution and TMB substrate must be kept warm at 37°C for 30 min. before use. When diluting samples and reagents, they must be mixed completely and evenly. The 96-well plate should not be dry at any time (drying will inactivate the active components on the plate).

1. Aliquot 0.1 ml per well of the 4000 pg/ml, 2000 pg/ml , 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml human NRG-1 standard solutions into the precoated 96-well plate. Add 0.1 ml of the sample diluent buffer into the control well (Zero well). Add 0.1 ml each of the properly diluted samples of human cell culture supernatant, serum or plasma to each empty well. See “Sample Dilution Guideline” for details.

   Notes:

   a. We recommend that each human NRG-1 standard solution and each sample is measured in duplicate.
   b. We recommend doing a pilot experiment using standards and a small number of samples to validate the assay procedure with the specific samples and optimize the appropriate sample dilution. Do not reuse tips and tubes to avoid cross contamination.

2. Seal the plate with the cover and incubate at 37°C for 90 min. Remove the cover, discard plate contents, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.
3. Add 0.1 ml of detection antibody working solution into each well and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01 M TBS or 0.01 M PBS, and each time leave the washing buffer in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 min. Repeat this process two additional times for a total of three washes. Note: For automated washing, aspirate all wells and wash three times with PBS or TBS buffer, overfilling wells with buffer each time. Blot the plate onto paper towels or other absorbent material.)
4. Add 0.1ml of prepared HRP conjugate working solution into each well and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01 M TBS or 0.01 M PBS, and each time leave washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 3 for plate washing method).
5. Add 90µl of prepared TMB substrate into each well and incubate plate at 37°C in dark for 25-30 min.

   Note: These guidelines are for reference only; the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the four most concentrated human NRG-1 standard solutions; the other wells show no obvious color.
6. Add 0.1 ml of stop solution into each well. The color changes into yellow immediately.
7. Read absorbance at 450 nm in a microplate reader within 30 min. after adding the stop solution.
8. Calculation: Relative $O.D._{450}$ = $O.D._{450}$ of each well – $O.D._{450}$ of Zero well. The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human NRG-1 concentration of the samples can be interpolated from the standard curve. **Note:** if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the original concentration in the sample. Take the volume change due to activation of NRG-1 into account.

Typical Data Obtained from Human NRG-1
(TMB reaction incubated at 37°C for 25 min.)

Concentration(pg/ml)	0.0	62.5	125	250	500	1000	2000	4000
O.D	0.031	0.095	0.175	0.299	0.596	1.008	1.651	2.311

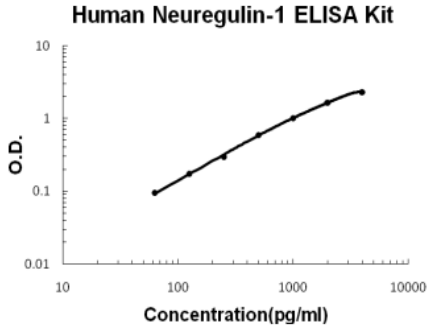

Figure: Standard Curve: This standard curves is for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

Neuregulin/Heregulin-1β (NRG-1β/HRG-1β), human recombinant (4711)
Neuregulin/Heregulin-1β Antibody (6650)
Heregulin-β2/Neuregulin-1 Antibody (6645)

FOR RESEARCH USE ONLY! Not to be used on humans.