BioAim Scientific Inc

Human NRG1-beta1 EasyTest$^{\text{TM}}$ ELISA Kit

Cat.No: 1010012

Instruction Manual

For research use only

TABLE OF CONTENTS

I. INTRODUCTION

Neuregulin 1 or NRG1 is a protein that in humans is encoded by the NRG1 gene. NRG1 is one of four proteins in the neuregulin family that act on the EGFR family of receptors. It is essential for the normal development of the nervous system and the heart.

Neuregulin 1 (NRG1) was originally identified as a 44-kD glycoprotein that interacts with the NEU/ERBB2 receptor tyrosine kinase to increase its phosphorylation on tyrosine residues.These isoforms include heregulins (HRGs), glial growth factors (GGFs) and sensory and motor neuron-derived factor (SMDF). The receptors for all NRG1 isoforms are the ERBB family of tyrosine kinase transmembrane receptors. Through their displayed interaction with ERBB receptors, NRG1 isoforms induce the growth and differentiation of epithelial, neuronal, glial, and other types of cells. The protein also has the putative ability to protect the brain from damage induced by stroke.

Neuregulin-1 (NRG-1) is necessary for cardiac development, structural maintenance, and functional integrity of the heart. NRG-1 and its receptor family ErbB can play a beneficial role in the treatment of chronic heart failure (CHF) by promoting survival of cardiac myocytes, improving sarcomeric structure, balancing Ca2+ homeostasis, and enhancing pumping function. Downstream effectors of NRG-1/ErbB, include cardiac-specific myosin light chain kinase (cMLCK), Protein Phosphatase type 1 (PP1), sarcoplasmic reticulum Ca2+-ATPase 2 (SERCA2), and focal adhesion kinase (FAK).

The BioAim Human NRG1-beta1 EasyTest™ ELISA kit can quantitatively measure NRG1-beta1 in human serum or plasma. It is a simple and rapid technology for the quantitation of antigen in a range of sample matrices. The whole process takes less than 1.5 hours with high accuracy and precision. EasyTest™ ELISA is faster and easier to perform than standard format ELISA with less reagent handling and fewer pipetting steps.

II. REAGENTS

1. Human NRG1-beta1 Microplate: 96 breakable wells (12strips x 8wells) coated with anti-human NRG1-beta1.
2. 20x Wash Buffer Concentrate: 1 Vial, 25 ml.
3. 5x Assay Diluent: 1vial, 15 ml.
4. Standards: 8µl/ vial, 2 vials, recombinant human NRG1-beta1.
5. BioAim human NRG1-beta1 Mix: 9µl/vial, 4 vials.
6. TBM Substrate solution: 1 Vial, 12 ml.
7. Stop Solution: 1 Vial, 8 ml of 0.2 M sulfuric acid.

III. STORAGE

1. The kit can be stored for up to 6 months at 2º to 8ºC from the date of shipment.
2. Standard can be stored at -20 ºC or -80 ºC. Use freshly prepared standard within 12 hours (stored at 2~8ºC).
3. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8ºC. Return unused strip to the pouch containing desiccant pack, reseal along entire edge and keep in 2~8ºC.
4. Avoid repeated freeze-thaw cycles.

IV. ADDITIONAL MATERIALS REQUIRED

1. Distilled or deionized water.
2. Precision pipettes, with disposable plastic tips.
3. Beakers, flasks, cylinders necessary for preparation of reagents.
4. Microplate washing device (multichannel pipette or automated microplate washer).
5. Microplate shaker.
6. Microplate reader capable of reading at 450 nm.

V. PRECAUTIONS

1. All reagents must be at room temperature (18ºC to 25ºC) before running assay.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. Do not use kit reagents beyond expiration date on label.
4. Do not expose kit reagents to strong light during storage or incubation.
5. Use disposable pipette tips for each transfer to avoid microbial contamination or cross contamination of reagents.
6. Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results.
7. Avoid contact of stop solution with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
8. Do not use TMB substrate solution if it has begun to turn blue.
9. Do not expose bleach to work area during actual test procedure because of potential interference with enzyme activity.

VI. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18~25°C) before use.
2. **Assay diluent**
   Dilute the concentrated assay diluent 1:5 with distilled water (e.g. 10ml plus 40ml).
3. **Wash Buffer**
   Dilute the concentrated wash buffer 1:20 with distilled water (e.g. 20ml plus 380ml).
4. **Sample**
   Levels of the target protein may vary among different specimens. Optimal dilution factors for each sample must be determined by the investigator.

The dilution scheme is only suggestion: the recommended dilution for serum and plasma is 1: 2.

5. Standard

a. Briefly spin standard vial before use. Add 392 µl 1x Assay Diluent to prepare a 200ng/ml standard. Gently vortex to mix.
b. Take 150 µl NRG1-beta1 standard into a tube; then add 350 µl 1x Assay Diluent to prepare a 60000 pg/ml stock standard solution.
c. Add 150 µl 1x Assay Diluent to 7 tubes. Label as 30000pg/ml, 15000pg/ml, 7500pg/ml, 3750pg/ml, 1875pg/ml, 937.5pg/ml and the last tube with 1x assay diluent is the blank as 0pg/ml.
d. Perform serial dilutions by adding 150 µl of each standard to the next tube and vortexing between each transfer (see figure below).

| 150µl | 150µl | 150µl | 150µl | 150µl | 150µl |

| 60000 Pg/ml | 30000 Pg/ml | 15000 Pg/ml | 7500 Pg/ml | 3750 Pg/ml | 1875 Pg/ml | 937.5 Pg/ml | 0 p/ml |
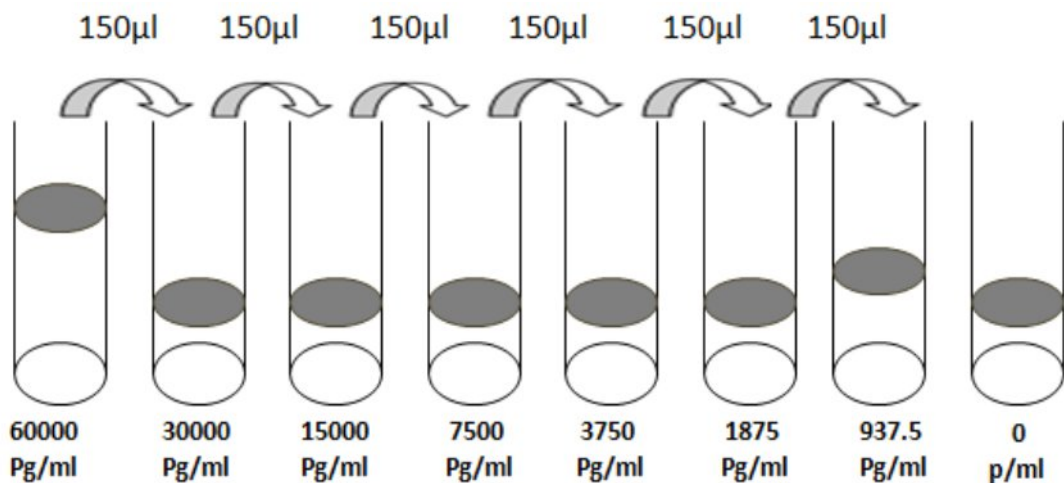
6. BioAim human NRG1-beta1 Mix

Within15minutes prior to use, briefly spin the vial. Add 1490 µl of 1x Assay diluent to the vial and mix by pipetting. A vial mix can be used for around 30 wells.

VII. ASSAY PROCEDURE

1. All reagents must be brought to room temperature (18-25°C) prior to use. Place the required number of microwells in the holder. It is recommended that all samples, standards, and blanks be run in duplicate.
2. Add 50 µl of 1x Assay Diluent into the blank wells.
3. Add 50 µl of each standard (*see reagent preparation step 5*) and samples into the designated wells. Gently shake/tap the plate for 5 seconds to mix.
4. Add 50 µl of BioAim NRG1-beta1 Mix into all wells, including the blank wells.
5. Cover wells with plate sealer and incubate at room temperature (18~25°C) for 1 hour with gentle shaking.
6. Decant or aspirate contents of wells. Wash wells by filling with at least 300 µl/well prepared wash buffer followed by decanting/aspirating. Soak wells in wash buffer for 30 seconds to 1 minute for each wash. Repeat wash 4 times for a total of 5 washes. After the last wash, blot plate on absorbent paper to remove residual buffer. Thorough washing at this step is very important, complete removal of liquid is required for proper performance.
7. Pipette 100 µl of TMB Substrate Solution to each well. Incubate plate for 15 minutes at room temperature in the dark with gentle shaking.
8. Add 50 µl of stop solution to each well.
9. Read absorbance at 450nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract the optical density readings at 570nm from readings at 450nm.

VIII. CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of duplicate standards, samples and controls. Subtract the average zero standard optical density.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the NRG1-beta1 concentration on log-log graph paper or using Sigma plot software. Draw a best fit curve through the points of the graph.
3. To determine the concentration of circulating NRG1-beta1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding NRG1-beta1 concentration.
4. A representative standard curve is shown below. This standard curve is for demonstration only. A standard curve must be run with each assay by operator.
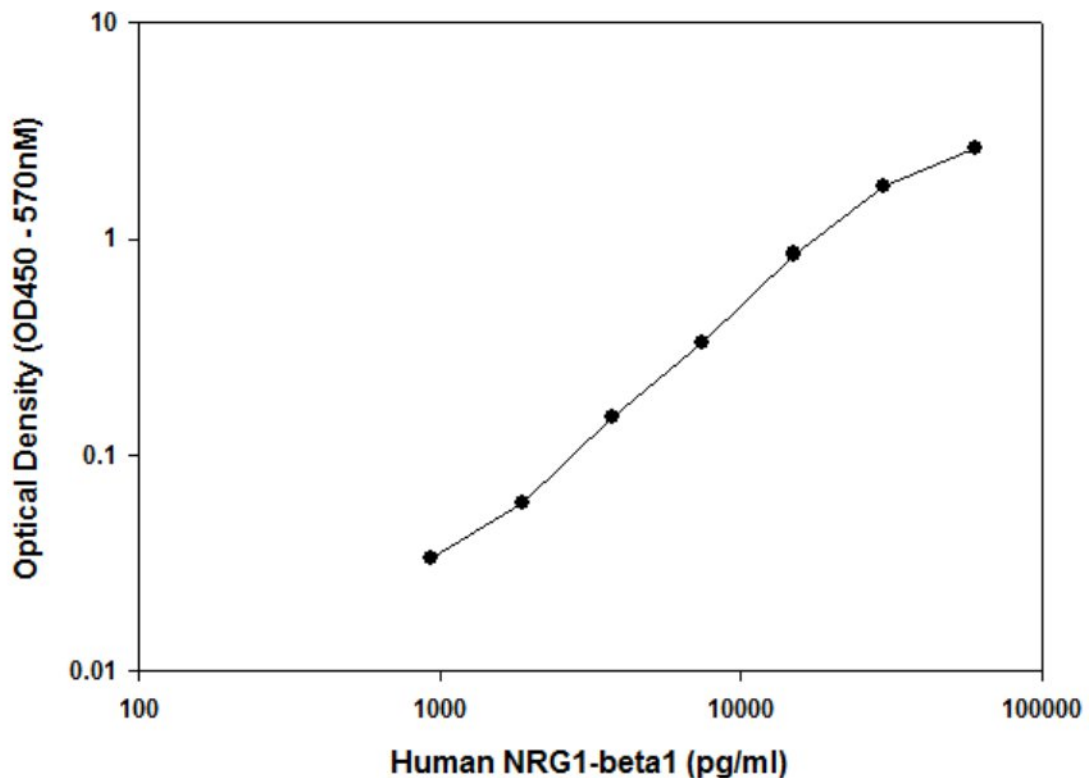
IX. PERFORMANCE

A. Sensitivity
The minimum detectable dose of NRG1-beta1 was determined to be 200pg/ml. This is defined as two standard deviations above the mean optical density of 20 replicates of the zero standards.

B. Recovery
Recovery was determined by spiking various levels of Human NRG1-beta1 into the diluted sample types listed below. Mean recoveries are as follows:

Sample Type	Average % recovery	Range %
Serum	123	118-131
Plasma	126	124-138

C. Linearity

Sample	Dilution	% of expected
Seum	1:2	95
	1:4	90
	1:8	96
Plasma	1:2	92
	1:4	83
	1:8	81

D. Specificity
No cross-reactivity was identified with the following cytokines: BDNF, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin, MCP-1, PDGF, RANTES, SCF, TGF-beta, TIMP-2, TNF-alpha, TNF-beta, and VEGF.

E. Reproducibility
Intra-Assay CV%: <10%
Inter-Assay CV%: <15%

X. REFERENCES

1. Holmes WE, etal. (May 1992) Science 256 (5060): 1205–10.
2. Orr-Urtreger A, etal. (March 1993). Proc. Natl. Acad. Sci. U.S.A. 90 (5): 1867–71.
3. Britsch S (2007). Adv Anat Embryol Cell Biol. Advances in Anatomy Embryology and Cell Biology 190: 1–65.
4. Stefansson H, etal. (2002). Am. J. Hum. Genet. 71 (4): 877–92.
5. Xu Z, etal. (2006). J. Cereb. Blood Flow Metab. 26 (4): 527–35.
6. Xu Y,etal. (2010). Adv. Pharmacol. Advances in Pharmacology 59: 31–51.
7. Kuramochi Y, etal. (2004). J. Biol. Chem. 279(49): 51141–7. .
8. Seguchi O, etal. (2007). J. Clin. Invest. 117 (10): 2812–24.
9. El-Armouche A, etal. (March 2003). FASEB J. 17 (3): 437–9

XI. Troubleshooting

Problem	Cause	Solution
1.Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. check pipettes; 2. Ensure briefly spin the vial of standard, take the right amount to dilution.
2. Low signal	1. Too brief incubation time 2. Inadequate reagent volumes or improper dilution	1. ensure adequate incubation time; 2. Check pipettes and ensure corrected preparation.
3. Large CV	Inaccurate pipetting	1. Check pipettes; 2. Accurately perform each step.
4.High background	1. Plate is insufficiently washed; 2. Wash buffer contamination	1.Follow the manual correctly; if using a plate washer, check that all ports are working functionally; 2. Prepare fresh buffer.
5. Low sensitivity	1.ELISA kit improper storage 2. Stop solution	1. Follow the manual to store each component correctly; 2. Add enough stop solution to each well.

Bioaim Scientific Inc

Unit 6, 27 Casebridge Court
Scarborough, ON, M1B 4Y4
Canada
Tel: 416-286-6868
www.bioaimscientific.com