DATA SHEET

BDNF (Human)
ELISA Kit
Catalog Number KA0329
96 assays

Introduction

Intended Use
For quantitive detection of human BDNF in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Background
Brain-derived neurotrophic factor (BDNF) is a prosurvival factor induced by cortical neurons that is necessary for survival of striatal neurons in the brain. It is a secreted protein with the molecular weight of 27.8kDa, consisting of 247 amino acids. It is known to promote neuronal survival and differentiation. BDNF shares substantial amino acid sequence identity with nerve growth factor (NGF). BDNF and neurotrophin-3 (NT-3) are two recently cloned neurotrophic factors that are homologous to NGF. mRNA products of the BDNF and NT-3 genes are detected in the adult human brain, suggesting that these proteins are involved in the maintenance of the adult nervous system.1 BDNF and other neurotrophins are critically involved in long-term potentiation (LTP). BDNF-mediated LTP is induced postsynaptically.2 BDNF has trophic effects on serotonergic (5-HT) neurons in the central nervous system.3 BDNF has an essential maintenance function in the regulation of anxiety-related behavior and in food intake through central mediators in both the basal and fasted state.4 It plays a role in treating breathing disorders such as respiratory insufficiency after spinal injury.5 The mature form of BDNF is identical in all mammals examined, and the gene encoding human BDNF to chromosome 11, band p13.6

Principle of the Assay
The human BDNF ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human BDNF specific-specific polyclonal antibodies were precoated onto 96-well plates. The human specific detection monoclonal antibodies (clone No. 35928.11) were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human BDNF amount of sample captured in plate.

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General Information
Materials Supplied
List of komponent

Component	Amount
Lyophilized recombinant human BDNF standard	10 ng/tube×2
One 96-well plate precoated with anti- human BDNF antibody	1
Sample diluent buffer	30 ml
Biotinylated anti- human BDNF, dilution 1:100	130 µl
Antibody diluent buffer	12 ml
Avidin-Biotin-Peroxidase Complex (ABC), dilution 1:100	130 µl
ABC diluent buffer	12 ml
TMB color developing agent	10 ml
TMB stop solution	10 ml

Storage Instruction
Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles (Shipped with wet ice.) Four months at 4°C and eight months at -20°C.

Materials Required but Not Supplied
_ Microplate reader in standard size.
_ Automated plate washer.
_ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
_ Clean tubes and Eppendorf tubes.
_ Washing buffer (neutral PBS or TBS).
Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na2HPO4 and 0.2g NaH2PO4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Precautions for Use

• Precautions
_ Before using Kit, spin tubes and bring down all components to bottom of tube.
_ Duplicate well assay was recommended for both standard and sample testing.
_ Don't let 96-well plate dry, dry plate will inactivate active components on plate.
_ In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37 ° C for 30 min before using.

Assay Protocol
Reagent Preparation
• Plate Washing
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 minutes. 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 PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.

• Reconstitution of the human BDNF standard: BDNF standard solution should be prepared no more than
2 hours prior to the experiment. Two tubes of BDNF standard (10ng per tube) are included in each kit.
Use one tube for each experiment.
a. 10,000pg/ml of human BDNF standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
b. 2000pg/ml of human BDNF standard solution: Add 0.2 ml of the above 10ng/ml BDNF standard solution into 0.8 ml sample diluent buffer and mix thoroughly.
c. 1000pg/ml→31.2pg/ml of human BDNF standard solutions: Label 6 Eppendorf tubes with 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 2000pg/ml BDNF 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 hours. The 10 ng/ml standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.
• Preparation of biotinylated anti-human BDNF antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
b. Biotinylated anti-human BDNF antibody should be diluted in 1:99 with the antibody diluent buffer and mixed thoroughly.
• Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume).
b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:99 with the ABC dilution buffer and mixed thoroughly

Sample Preparation
Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C.
Avoid repeated freeze-thaw cycles.
• Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
• Serum: Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
• Plasma: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 15 min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8 ° C at 10000 x g. Analyze immediately or aliquot and store samples at -20°C.

Sample Dilution Guideline
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 in the standard curve. Dilute the sample using the provided diluent buffer.

The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.
_ High target protein concentration (20-200 ng/ml). The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
_ Medium target protein concentration (2-20 ng/ml). The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
_ Low target protein concentration (31.2-2000 pg/ml). The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
_ Very Low target protein concentration (≤31.2 pg/ml). No dilution necessary, or the working dilution is 1:2.

Assay Procedure
The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard BDNF detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of BDNF amount in samples.
1. Aliquot 0.1ml per well of the 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml human BDNF standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See "Sample Dilution Guideline" above for details. We recommend that each human BDNF standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human BDNF antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C for 15-20min (shades of blue can be seen in the wells with the four most concentrated human BDNF standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

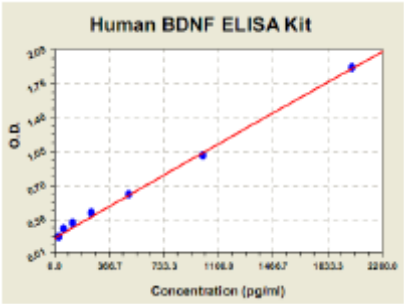
Data Analysis

Calculation of Results

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the 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 Mouse Havcr1 concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.



Performance Characteristics
• Range 31.2 pg/ml-2000 pg/ml
• Sensitivity < 2 pg/ml
• Specificity No detectable cross-reactivity with any other cytokine.

Resources References
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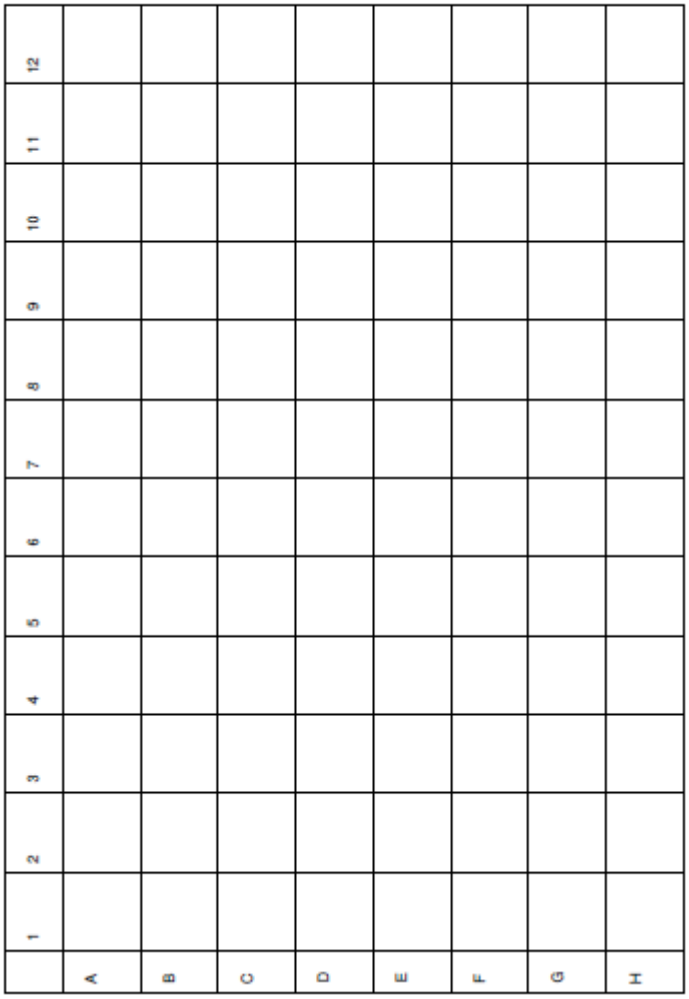
Plate Layout



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