DATA SHEET

Human BDNF ELISA
Catalog # LF-EK50005 (1 kit)

Catalog # LF-EK50006 (4 kits bundle)
Catalog # LF-EK50007 (10 kits bundle)
Catalog # LF-EK50008 (20 kits bundle)

Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative Detection of human BDNF

For research use only
Not for diagnostic or therapeutic purposes

1. Introduction

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

2. Principles of Method

AbFrontier's 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 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.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

3. Intended Use

The AbFrontier human BDNF ELISA kit is to be used for the in vitro quantitative determination of human BDNF in human serum, human plasma, cell lysate and buffered solution. The assay will recognize both native and recombinant human BDNF.

This kit has been configured for research use only and is not to be used in diagnostic procedures.

4. Storage and Stability

All kit components of this kit are stable at 2 to 8℃. Any unused reconstituted standard should be discarded or frozen at -70℃. Standard can be frozen and thawed one time only without loss of immunoreactivity.

5. Chemical Hazard

- Stop solution: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.
- All reagents containing Sodium Azide also contain Thimerosal as a preservative. Thimerosal contains Hg thus should be handled with great care.

6. Kit Contents

Contents	Number	Volume
96 Well Plate	1 (in aluminum foil bag with desiccant)	
Standard Protein	2	10ng/tube
Secondary Antibody	1	130 ul
Avidin-Biotin-Peroxidase Complex (ABC)	1	130 ul
Sample diluent Buffer	1	30 ml
Antibody diluent buffer	1	12 ml
ABC diluent buffer	1	12 ml
TMB color developing agent	1	10 ml
TMB stop solution	1	10 ml

①96 Well Plate
: Human BDNF microtiter plate, one plate of 96 wells.
A plate using break-apart strips coated with a monoclonal antibody specific to human BDNF.
②Standard Protein
: Recombinant human BDNF.
③Secondary Antibody
: Biotin labeled anti human BDNF antibody.
④AV-HRP
: Avidin-Biotin-Peroxidase Complex (ABC)
⑤Substrate (Stabilized chromogen)
: Tetramethylbenzidine (TMB) solution
⑥Stop Solution
: 1 N solution of sulfuric acid (H2SO4)

Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. 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℃ for 30 min before using.

7. Materials Required But Not Provided

①Microtiter plate reader in standard size.
②Automated plate washer.
③Distilled or deionized water
④ Calibrated, adjustable precision pipettes, preferably with disposable plastic tips (A manifold multi-channel pipette is desirable for large assays.)
⑤Data analysis and graphing software
⑥Vortex mixer
⑦Polypropylene tubes for diluting and aliquoting standard
⑧Absorbent paper towels
⑨Calibrated beakers and graduated cylinders of various sizes
⑩Washing 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.

8. Reagent Preparation

1) 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.

2) Sample Preparation and Storage

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.

o **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C

o **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.

o **Plasma**: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 15 min at 2-8℃ 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.

3) 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.**

o **High target protein concentration (20-200ng/ml).** The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.

o **Medium target protein concentration (2-20ng/ml).** The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.

o **Low target protein concentration (31.2-2,000pg/ml).** The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.

o **Very Low target protein concentration (≤31.2pg/ml).** No dilution necessary, or the working dilution is 1:2.

4) Reagent Preparation and Storage

A. Reconstitution of the human BDNF standard：BDNF standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of human 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 human 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.

B. 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.

C. 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.

9. 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.

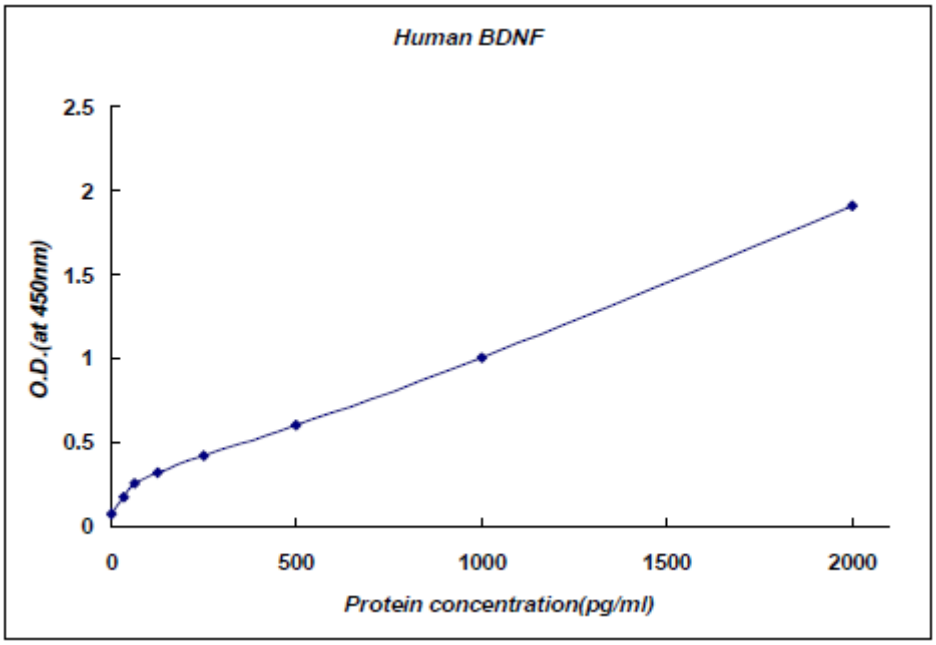
1. Aliquot 0.1ml per well of the 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml human BDNF standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each

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 15-20 min (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. 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 human BDNF 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.
Summary
1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C for 10-15 min.
5. Add TMB stop solution and read.

10. Characteristics
1) Typical result
Typical Data Obtained from Human BDNF (TMB reaction incubate at 37℃ for 16 min)

Standard human BDNF (pg/ml)	Optical Density (at 450nm)
0	0.071
31.2	0.170
62.5	0.254
125	0.321
250	0.419
500	0.603
1000	1.006
2000	1.911

Typical Human BDNF ELISA kit Standard Curve
This standard curve was generated at AbFrontier for demonstration purpose only. A standard curve must be run with each assay.



2) Sensitivity: < 2pg/ml
3) Specificity: No detectable cross-reactivity with any other cytokine.

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11. Troubleshooting

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much AV-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20~30 ℃ before performing assay
Too much signal – whole plate turned uniformly blue	• Insufficient washing – unbound AV-HRP remaining	• Increase number of washes carefully
	• Too much AV-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual AV-HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

12. Reference

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