4. Linearity

Two different patient samples were diluted with the sample diluent to 1:200, 1:400 and 1:800. BDNF values were calculated and results were corrected with the dilution factor.

	Original Value	Percentage of Recovery		
Serum	pg/mL	1/200	1/400	1/800
1	19230.8	96.7	97.5	90.0
2	34855.7	99.3	101.1	103.9

ADDITIONAL INSTRUCTIONS

1. Do not perform standard or sample dilutions within the experimental well to avoid damaging the plate.
2. It is recommended that at least duplicate measurements, if not triplicate for each standard and sample dilution be performed for best evaluation of the results.
3. Dilute samples to a BDNF concentration that falls within the range of the standard curve. Do not extrapolate absorbance readings for best results.
4. Avoid touching the inside surface of the plate wells with the pipette tip or sharp objects.
5. Complete removal of liquid from the wells at each step is essential for reliable results. However, avoid letting the wells dry out at any time. Complete the assay in one session if possible.
6. Add TMB and the stop solution to the wells in the same order to all wells.
7. Ensure that all bubbles are removed and that the bottom of the plate is optically clean prior to taking the absorbance reading for best results.
8. All incubations are at room temperature (20-25˚C, 70-75˚F) in our laboratories.

MANUAL WASHING INSTRUCTIONS

Proper emptying and washing of the plate is crucial for low backgrounds, consistent readings, and the reduction of non-specific binding. For manual plate washing, we recommend the following procedure:

a) To remove liquid from the wells, place the plate on the palm of the hand and quickly invert the plate over the sink. Forcefully move the arm downwards and stop abruptly to force the liquid out of the wells. When done correctly, this technique should prevent liquid from getting onto the fingers or onto the outside of the microplate wells
b) Blot and forcefully tap the microplate against clean paper towels for 3-5 times.
c) Wash the wells by pipetting 200 µL of wash buffer into each well and empty the wells as described in step a-b)
d) Repeat this procedure for a total of 3 times for each wash cycle.

REFERENCES:

1. Tsuchimine et al., Preanalysis Storage Conditions Influence the Measurement of Brain-Derived Neurotrophic Factor Levels in Peripheral Blood. Neuropsychobiology. 2014 April; 69(2): 83-87.
2. Polacchini et al., A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays. Scientific Reports. 2015 December; 5:17989 | DOI: 10.1038/srep17989.

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HUMAN BDNF ELISA

Catalog No.: Biosensis: BEK-2211-1P-CE | Calbiotech: BD321G (96 Tests)

INTENDED USE

The purpose of this kit is the quantitative determination of mature human BDNF in human serum, or citrate treated plasma samples only when used as directed.

SUMMARY AND EXPLANATION

Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophin family of growth factors that play an important role in a variety of physiological functions, for instance mediating neuronal survival and apoptosis, maintaining synaptic plasticity and regulating synaptic transmission. Altered BDNF levels in the central nervous system and blood are implicated in a variety of neurodegenerative diseases such as amyotrophic lateral sclerosis, neuropathic pain and Alzheimer's disease.

PRINCIPLE OF THE TEST

The Biosensis BDNF Rapid™ enzyme-linked immunosorbent assay (ELISA) Kit is a sandwich ELISA that allows the preferential quantification of mature BDNF in less than 3 hours. This kit consists of a pre-coated mouse monoclonal anti-BDNF capture antibody, a biotinylated anti-BDNF detection antibody and horseradish peroxidase (HRP)-conjugated streptavidin. The addition of a substrate (3,3',5,5'- tetramethylbenzidine, TMB) yields a colored reaction product which is directly proportional to the concentration of BDNF present in samples and protein standards. This BDNF ELISA kit employs a recombinant human BDNF standard approved by the World Health Organization (WHO, www.nibsc.org). This kit is suitable to measure mature BDNF in human serum and suitably treated plasma samples only. The antibodies used in this ELISA kit bind epitopes within the mature domain of the protein and therefore recognize the mature as well as the pro-form of BDNF. However, cross-reactivity to the full-length proBDNF protein is low.

MATERIALS PROVIDED | Biosensis: BEK-2211-1P-CE | Calbiotech: BD321G (1 plate)		96 Tests
1.	BDNF antibody coated 96 well microplate strips	1, 96w set:12x8
2.	Assay Diluent A* (ready to use)	2 x 25 mL
3.	Recombinant BDNF Standard, (freeze-dried)	2 x 1000 pg
4.	BDNF Detection antibody (ready to use)	1 x 12 mL
5.	Streptavidin-HRP (ready to use)	1 x 12 mL
6.	Wash Buffer (20x), 1 bottle	1 x 25 mL
7.	TMB Substrate (1x)	1 x 12 mL
8.	Stop Solution (1x)	1 x 12 mL
9.	Plate Sealer	4

*The assay diluent provided in this kit is suitable for measuring BDNF in serum or plasma. See SPECIMEN COLLECTION AND HANDLING section for the minimum required sample dilutions

REAGENT STORAGE & STABILITY

Reagent	Storage & Stability
ELISA kit as supplied	24 months at 2-8ºC
Reconstituted Standard	Use on same day; aliquot unused standard to prevent multiple freeze-thaw cycles and store at -20ºC for 2 weeks.
Detection Ab & HRP Conjugate (1X)	30 months at 2-8ºC
Diluted Wash Buffer	36 months at 2-8ºC

MATERIALS NOT PROVIDED

1. Precision pipettes
2. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450nm
4. Flat-head Vortex mixer
5. Plate shaker
6. Graph paper

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. This kit is intended for the quantitation of BDNF in human serum, or suitably treated human plasma samples only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate
6. Some test reagents contain chemicals and chemical mixtures which are considered hazardous. Please observe good laboratory practices and handling methods when using components of this assay.
7. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND HANDLING

Serum

- Allow the serum to clot in a serum separator tube (about 30 minutes to 4 hours) at room temperature.
- Centrifuge at approximately 1,000 x g for 15 minutes
- Analyze the serum immediately or aliquot and store frozen at -20°C to -80°C
- Dilute serum samples with Assay Diluent A to measure BDNF concentrations
- For human serum samples, a minimum dilution of 1/50 – 1/100 is required

Plasma

- Collect plasma using citrate as anticoagulant, other coagulants have not been qualified for use.
- Centrifuge for 15 min at 2-8°C at 1,500 x g within 30 minutes of collection
- For eliminating the platelet effect we suggest further centrifugation for 10 min at 2-8°C at 10,000 x g
- Analyze immediately or aliquot and store samples at -20°C to -80°C
- Dilute plasma samples at least 1/20 with Assay Diluent A to measure BDNF concentrations.

Note: Because of the release of BDNF from platelets, serum and plasma BDNF levels in humans will vary depending on temperature, storage time and anti-coagulant used. Serum BDNF levels are less variable than plasma BDNF concentrations and reach stable peak levels after 1 hour of incubation (Tsuchimine et al., 2014). Thus, to prevent sample variation, strict adherence to consistent sample preparation procedures among samples and study groups are highly recommended.

Moreover, for unknown concentrations of BDNF in samples, it is important to perform several dilutions of the sample to allow the BDNF concentration to fall within the range of the BDNF standard curve (7.8-500 pg/mL). Typical normal human serum values are 10-30 ng/mL; Plasma values are more variable but range from 2-20 ng/mL in most cases if collected quickly.

REAGENT PREPARATION

20X Wash Buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 mL, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

Preparation of BDNF Standard

- Reconstitute the lyophilized antigen standard with 1 mL of the same diluent used for preparing sample dilutions.
- Label the vial with the reconstituted BDNF standard as "1000 pg/mL"; vortex and let stand for 15 minutes.
- Dilute the 1000 pg/mL BDNF standard 1:2 (eg. 500 µL of 1000 pg/mL standard + 500 µL diluent); label this tube "500 pg/mL".
- Note: 500 pg/mL is the highest concentration of the BDNF standard curve.

In order to generate a BDNF standard curve, perform a 1:2 serial dilutions down to 7.8 pg/mL. The volumes used for the dilution series depends on the number of repeats per BDNF concentration. For triplicate measurement (100 µL per well) of each BDNF standard concentration, we recommend this procedure:

1. Label 6 tubes with "250 pg/mL", "125 pg/mL", "62.5 pg/mL", "31.3 pg/mL", "15.6 pg/mL" and "7.8 pg/mL", respectively
2. Aliquot 400 µL of the sample diluent into each tube
3. Take 400 µL from the "500 pg/mL" tube and transfer to the "250 pg/mL" tube
4. Pipet up and down and vortex to mix to avoid foaming, use only a very brief vortex
5. Repeat steps 3 and 4 for each consecutive concentration until the last tube "7.8 pg/mL" is prepared and mixed well.
6. Standard solution should be prepared no more than 2 hours prior to the experiment. The working standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 24 hours. Avoid repeated freeze-thaw cycles.

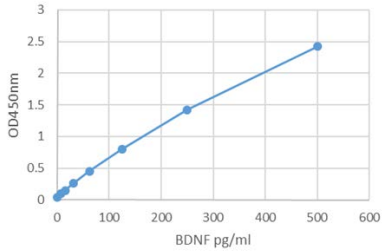
PROCEDURE:

All steps are performed at room temperature (20-25°C, 70-75°F).

1. Add 100 µL of diluted BDNF standards, samples and blank (sample diluent only) to the pre-coated microplate wells
2. If available, include a negative and positive control sample in the assay procedure.
3. Seal the plate (e.g. with plate sealer or parafilm) and incubate the plate with moderate shaking for 60 minutes.
4. Discard the solution inside the wells and perform 3 washes with 1x wash buffer (350 µL per well). See the ADDITIONAL INSTRUCTIONS section for a detailed description of the washing procedure (MANUAL WASHING INSTRUCTIONS).
5. Add 100 µL of the detection antibody into each well.
6. Seal the plate (e.g. with plate sealer or parafilm) and incubate the plate with moderate shaking for 30 minutes.
7. Discard the solution inside the wells and wash as described in step 4
8. Add 100 µL of the streptavidin-HRP conjugate into each well
9. Seal the plate (e.g. with plate sealer or parafilm) and incubate the plate with moderate shaking for 30 minutes.
10. Discard the solution inside the wells and wash as described in step 4
11. Add 100 µL of TMB into each well and incubate plate at room temperature for 15 minutes without shaking in the dark.
12. Stop the reaction by adding 50µL of the stop solution into each well. Visible blue color will change to yellow. Read the absorbance at 450 nm on a plate reader. Note: Color will fade over time; hence, we recommend plate to be read within 5 minutes after adding the stop solution or no longer than 30 minutes after addition.

Example of a Standard Curve

	Conc. pg/mL	OD 450nm
Std 1	0	0.05
Std 2	7.8	0.10
Std 3	15.6	0.15
Std 4	31.3	0.26
Std 5	62.5	0.45
Std 6	125	0.80
Std 7	250	1.42
Std 8	500	2.42



Calculation of Results

1. Average the readings for each BDNF standard concentration, blank and sample.
2. Plot a standard curve with the BDNF standard concentration on the x-axis and the OD at 450 nm on the y-axis.
3. If values for the BDNF standards are adjusted for background absorbance, then subtract the blank value from the OD450 of the samples as well.
4. Use appropriate software to reduce the data and generate a four-parameter logistic curve-fit. Do not use linear regression.
5. Perform a regression analysis to calculate the concentration of BDNF in the samples. Multiply the result by the sample dilution factor.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

The sensitivity was determined by calculating the mean + 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean pg/mL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	1.11	0.745	2.6

2. Correlation with a Reference ELISA kit:

Serum samples were tested by this ELISA kit and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.99	0.93	11.2

3. Precision

Intra-Assay

Serum	No. of Replicates	Mean pg/mL	Standard Deviation	Coefficient of Variation (%)
1	16	8.6	0.71	8.26
2	16	60.2	3.00	4.98
3	16	215.9	8.44	3.91

Inter-Assay

Serum	No. of Replicates	Mean pg/mL	Standard Deviation	Coefficient of Variation (%)
1	16	8.7	0.73	8.4
2	16	59.7	2.98	5.0
3	16	217.1	10.52	4.8