BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plate

Human BDNF

(free-form)

Cat. No. 446607 1 Plate

ELISA Kit for Accurate Quantitation of Human Free BDNF from Cell Culture Supernatant, Serum, and Plasma

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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Introduction:

Brain-derived neurotrophic factor (BDNF) is the second member of NGF family of neurotrophic factors (neurotrophins), which includes nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). BDNF shares approximately 55% amino acid identity with other family members. The amino acid sequence of mature BDNF is identical in all mammalian species examined to date. Mature BDNF promotes the survival and differentiation of selected neuronal populations of the peripheral and central nervous systems during development. BDNF participates in axonal growth, pathfinding, and in the modulation of dendritic growth and morphology. BDNF is also a major regulator of synaptic transmission and plasticity at adult synapses in many regions of the central nervous system (CNS). High levels of expression of BDNF have been detected in the hippocampus, cerebellum, fetal eye, and placenta. BDNF binds with high affinity and specifically activates the TrkB tyrosine kinase receptor. BDNF is also associated with multiple diseases such as Schizophrenia, Rett syndrome, obsessive compulsive disorder, Huntington's disease, eating disorders, depression, dementia and Alzheimer's disease. For example, BDNF level is decreased in Alzheimer's disease patients' serum samples.

The BioLegend LEGEND MAX™ Human BDNF Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit includes a 96-well strip plate that is pre-coated with a mouse monoclonal anti-human BDNF capture antibody. The detection antibody is a biotinylated mouse monoclonal anti-human BDNF antibody. This kit is specifically designed for the accurate quantitation of the free-form human BDNF from cell culture supernatant, serum, plasma, and other biological fluids. It has been analytically validated with ready-to-use reagents.

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Materials Provided:

Description	Quantity (1 plate)	Volume (per bottle)	Part #
Anti-human BDNF Precoated 96-well Strip Microplate	1 plate		750000195
Human BDNF Detection Antibody	1 bottle	12 mL	750000196
Human BDNF Standard	1 vial	lyophilized	750000197
Avidin-HRP	1 bottle	12 mL	77897
Assay Buffer A	1 bottle	25 mL	78232
Assay Diluent C	1 bottle	12 mL	750000239
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	76335
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Polypropylene tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate Strips</td><td>If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.</td></tr>
<tr><td>Standard</td><td>The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Detection Antibody</td><td rowspan="7">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Avidin-HRP</td></tr>
<tr><td>Assay Buffer A</td></tr>
<tr><td>Assay Diluent C</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid and is corrosive. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

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Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 20 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 20 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human BDNF Standard by following the instructions described in LEGEND MAX™ Kit Lot-Specific Certificate of Analysis/LEGEND MAX™ Kit Protocol enclosed in the kit. Allow the reconstituted standard to sit at room temperature for 15 to 20 minutes, then briefly vortex to mix completely.
3. In general, serum samples require a 10-fold dilution and other sample types are analyzed without dilutions. If dilutions are required, use Assay Buffer A as the sample diluent.

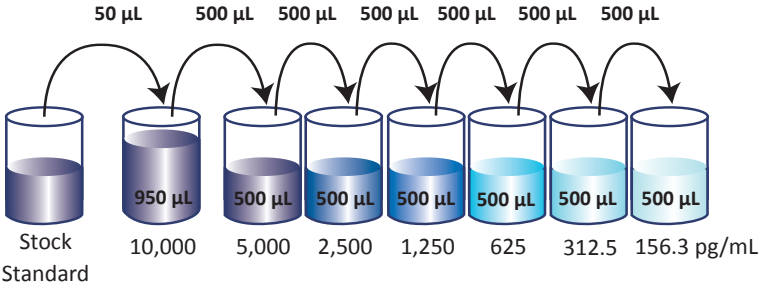
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 1,000 µL of the 10,000 pg/mL top standard by adding 50 µL of the standard stock in 950 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 10,000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the Human BDNF standard concentrations in the tubes are 10,000 pg/mL, 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 312.5 pg/mL, and 156.3 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on clean, absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of Assay Diluent C to each well that will contain the standard dilutions or samples.

6. Add 50 µL of standard dilutions or samples to the appropriate wells.

7. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with shaking.

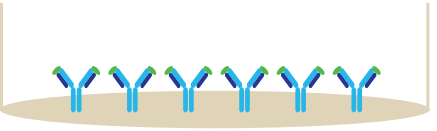
8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

9. Add 100 µL of Human BDNF Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour with shaking.

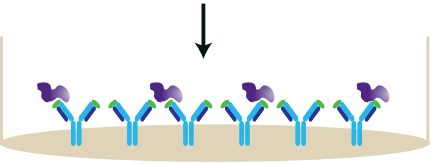
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Avidin-HRP solution to each well, seal and incubate the plate at room temperature for 30 minutes with shaking.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100 µL of Substrate Solution F to each well and incubate at room temperature for 15 to 20 minutes in the dark. Wells containing human BDNF should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm immediately. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

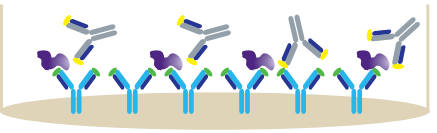
1. Wash 4 times
   Add 50 µL Assay Diluent C to standard wells and sample wells



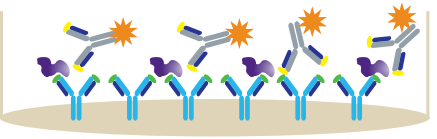
2. Add 50 µL diluted standards or samples,
   Incubate 2 hr, RT, shaking



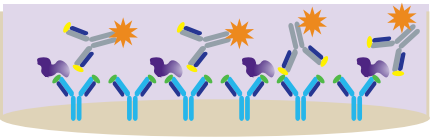
3. Wash 4 times
   Add 100 µL Detection Antibody solution
   Incubate 1 hr, RT, shaking



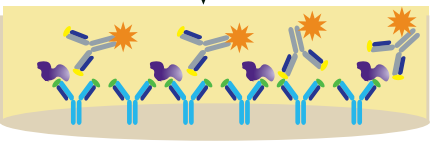
4. Wash 4 times
   Add 100 µL Avidin-HRP solution
   Incubate 30 min, RT, shaking



5. Wash 5 times
   Add 100 µL Substrate Solution F
   Incubate 15 to 20 min, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

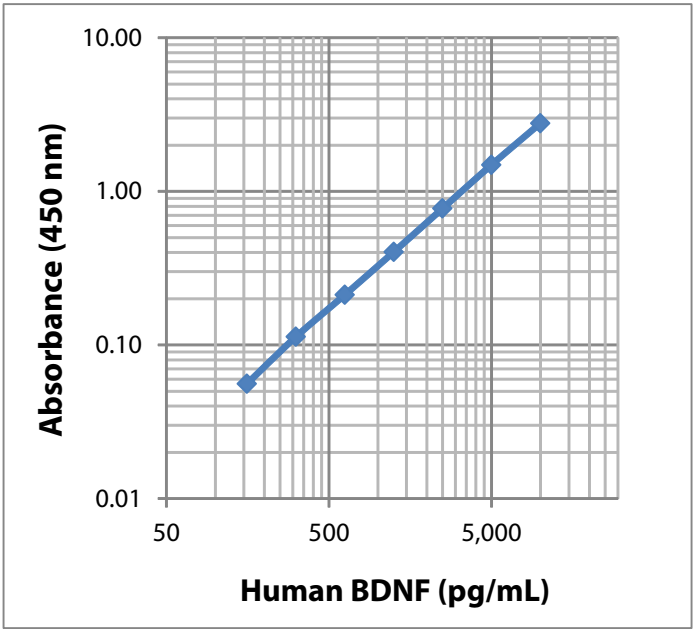
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with protein concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown protein concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the protein concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross-reactivity was observed when this kit was used to analyze the following recombinant proteins, each at 50 ng/mL.

Human	βNGF, CNTF, GDNF, IFN-γ, IGF-I, IGF-II, NGFR, NT-3, NT-4, TrkB (NTRK2), PDGF-AA, PDGFRB FC, PlGF-1, TGF-α, TGF-β1, TGF-β2, TGF-β3, TNF-α, VEGF-121, VEGF-B167, VEGF-D, VEGFR1, VEGFR2 Fc Chimera

TrkB interferes at all levels when various concentrations of recombinant human TrkB were added to a sample of known BDNF concentration (5,000 pg/mL).

Concentration of Human TrkB	BDNF: TrkB Molar Ratio	% Interference
234.3 pg/mL	1:0.01	8.9
3,750 pg/mL	1:0.2	19.1
15,000 pg/mL	1:0.5	50.8
30,000 pg/mL	1:1	71.5
60,000 pg/mL	1:2	87.2
240,000 pg/mL	1:10	95.7

Sensitivity: The average minimum detectable concentration is 27.5 pg/mL.

Recovery: Recombinant human BDNF (4,800, 1,200, and 300 pg/mL) was spiked into human samples, then analyzed with the LEGEND MAX™ Human BDNF ELISA Kit.

Sample Type	N	% Recovery
Serum	15	98.7
Citrate Plasma	10	47.5
EDTA Plasma	10	48.5
Heparin Plasma	10	65.0

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Linearity: Human serum samples containing high levels of human BDNF or plasma samples spiked with recombinant human BDNF were diluted to produce samples within the dynamic range, and then assayed with the kit to determine the dilutional linearity.

Sample Type	N	% Linearity
Serum	15	99.9
Citrate Plasma	10	160.9
EDTA Plasma	10	200.1
Heparin Plasma	10	198.9

Intra-Assay Precision: Two serum samples were spiked with different recombinant human BDNF concentrations and tested with 16 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	1701.8	382.5
Standard Deviation	213.6	48.9
% CV	12.6	12.8

Inter-Assay Precision: Two human samples were spiked with different concentrations of recombinant human BDNF and assayed in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	1817.3	349.2
Standard Deviation	235.8	58.1
% CV	16.6	13.0

Normal Human Biological Samples:
Normal Ranges: 57 human serum and plasma (Citrate, EDTA and Heparin) samples from human donors were tested for endogenous human BDNF.

Sample Type	N	% Detectable	Min (pg/mL)	Max (pg/mL)	Median (pg/mL)
Serum	24	100	6017.9	54421.4	30661.2
Citrate	11	100	30.3	3826.3	533.0
EDTA	11	100	117.6	2520.1	404.2
Heparin	11	100	443.6	3698.2	1262.7

Cell Supernatant: Peripheral blood mononuclear cells (PBMC) (1×10^6 cells/mL) from different donors were cultured and stimulated under different conditions. The cell supernatants were collected and assayed for human BDNF.

Donor ID, Stimulation Condition, Day collected	Concentration (pg/mL)
Donor #1, Unstimulated, Day 1	359.9
Donor #2, Unstimulated, Day 2	162.9
Donor #3, Unstimulated, Day 1	513.2
Donor #3, 50 ng/mL PMA, Day 1	377.3
Donor #3, 50 ng/mL LPS, Day 1	357.6

Non-human Biological Samples:

As the amino acid sequence of mature BDNF is identical in all sequenced mammalian species, serum or plamsa samples from non-human mammalian species were tested for endogenous BDNF.

Sample Type	N	Detectable	Concentration (pg/mL)
Mouse Serum	4	No	
Mouse Citrate Plasma	4	No	
Mouse EDTA Plasma	4	No	
Mouse Heparin Plasma	4	No	
Rat Serum	4	Yes	161.5 - 583.8
Donkey Serum	1	Yes	355.9
Equine Serum	1	Yes	176.5
Goat Serum	2	No	
Porcine Serum	1	Yes	74.6
Bovine Serum	1	No	
Rabbit Serum	1	No	
Moneky Serum	3	Yes	1759.5 - 2313.4

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Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
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