



Human BDNF ELISA Kit

Catalog number: **NR-E10076** (96 wells)

The kit is designed to quantitatively detect the level of **Human BDNF** in cell culture supernatant, serum, plasma and other suitable sample solution.

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before using this product, please read this manual carefully; After reading the subsequent contents of this manual, please note the following specially:

The operation should be carried out in strict accordance with the provided instructions.

Store the unused strips in a sealed foil bag at 2-8°C.

Always avoid foaming when mixing or reconstituting protein solutions.

Pipette reagents and samples into the center of each well, avoid bubbles.

The samples should be transferred into the assay wells within 15 minutes of dilution.

We recommend that all standards, testing samples are tested in duplicate.

Using serial diluted sample is recommended for first test to get the best dilution factor.

If the blue color develops too light after 15 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).

Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.

Avoid using the suction head without extensive wash.

Do not mix the reagents from different batches.

Stop Solution should be added in the same order of the Substrate Solution.

TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

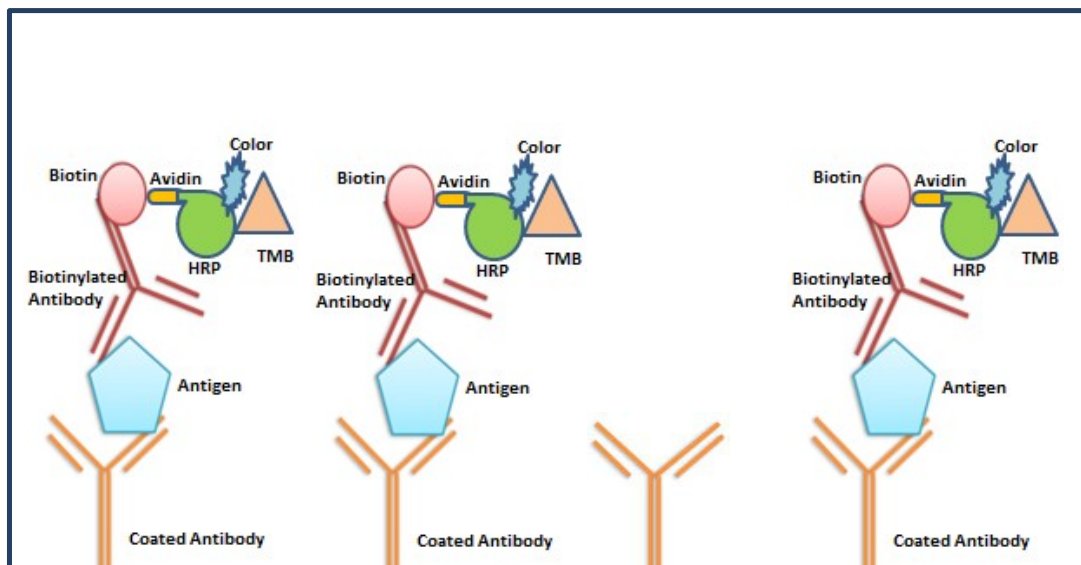
Intended use

The kit is used to quantify the **Human BDNF** in serum, plasma, body fluids, tissue lysate or cell culture supernatant.

Standard range	23.4--1500 pg/ml
Sensitivity	2.0 pg/ml
Assay time	4 hours
Validity	Six months
Store at	2-8 °C

Assay principle

This **Human BDNF** ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. **Human BDNF** specific antibody has been precoated onto 96-well plate. The test samples and the biotinylated **Human BDNF** specific detection antibody are added to the wells subsequently and then followed by washing the plate. Streptavidin-HRP is added and unbound conjugates are washed away with Wash Buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic Stop Solution. The density of yellow is proportional to the **Human BDNF** amount of sample captured in plate.



Materials supplied

- | | |
|---|------------------------|
| 1. Human BDNF standard: | 1.5 ng/vial ×2. |
| 2. 96-well plate pre-coated with anti- Human BDNF Ab: | 1. |
| 3. Sample diluent buffer: | 12 ml × 2. |
| 4. Detection antibody: | (dilution 1:180) 60 µL |
| 5. Streptavidin-HRP: | (dilution 1:40) 300 µL |
| 6. Antibody diluent buffer: | 12 ml. |
| 7. Streptavidin-HRP diluent buffer: | 12 ml. |
| 8. TMB developing agent: | 12 ml × 2. |
| 9. Stop Solution: | 12 ml. |
| 10. 20 × Wash Buffer: | 25 ml. |
| 11. Plate sealer | 1. |
| 12. Package insert | 1. |

Materials required but not supplied

37°C incubator.

Standard plate reader capable of measuring absorbance at 450 nm.

Adjustable pipettes and disposable pipette tips.

Multi-channel pipettes, manifold dispenser or automated microplate washer.

Distilled water.

Absorbent paper.

Materials used for sample preparation.

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.

Cell culture supernatant, 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 4hours) 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 as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C. EDTA and citrate are not recommended as the anticoagulant.

Reagent Preparation

Standard

Human BDNF: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (1.5 ng /vial) are included in each kit. Use one tube for each experiment.

1500 pg/ml → 23.4 pg/ml of Human BDNF standard solutions:

Add 0.75 ml of sample diluents into one standard tube with 1.5 ng Human BDNF. Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 1500 pg/ml standard solution.

Label 7 Eppendorf tubes with 1500 pg/ml, 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.75 pg/ml, 46.85 pg/ml, 23.4 pg/ml respectively. Transfer 0.4 mL of 1500 pg/ml standard solution into 1500 pg/ml tube. Then make 2-fold serial dilution from 1500 pg/ml to 23.4 pg/ml in seven 1.5 ml tubes.

Make sure each tube has ≥ 300 μ l of standard.

Note: The standard solutions are best used within 2 hours.

Preparation of biotinylated anti- Human BDNF antibody working solution

The solution should be prepared no more than 2 hours prior to the experiment.

The total volume should be: 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume).

Biotinylated anti-Human BDNF detection antibody should be diluted in 1:180 with Antibody diluent buffer and mixed thoroughly.

Preparation of Streptavidin-HRP working solution

The solution should be prepared no more than 1 hour prior to the experiment.

The total volume should be: 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume).

Streptavidin-HRP should be diluted in 1:40 with Streptavidin-HRP diluent buffer and mixed thoroughly.

Wash Buffer

If crystals have formed in the 20 × wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Dilute 25 ml Wash Buffer Concentrate (20 ×) to a total volume of 500ml with distilled water.

Assay procedures

Bring all reagents to room temperature before use. **Human BDNF** Standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of **Human BDNF** concentration in samples.

1. Add 100 µl of sample or standards per well. Add 0.1ml of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate 90 minutes at 37°C.
Note: We recommend that each **Human BDNF** standard solution and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µl of the Detection Antibody working solution to each well. Cover with a new adhesive strip and incubate 60minutes at 37°C.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at 37°C.
6. Repeat the aspiration/wash as in step 2 for five times.
7. Add 200µl of TMB developing agent to each well. Cover and incubate for 50-70 minutes at 37 °C (Protect from light. Do not over-develop).
8. Add 100µl Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Result calculation

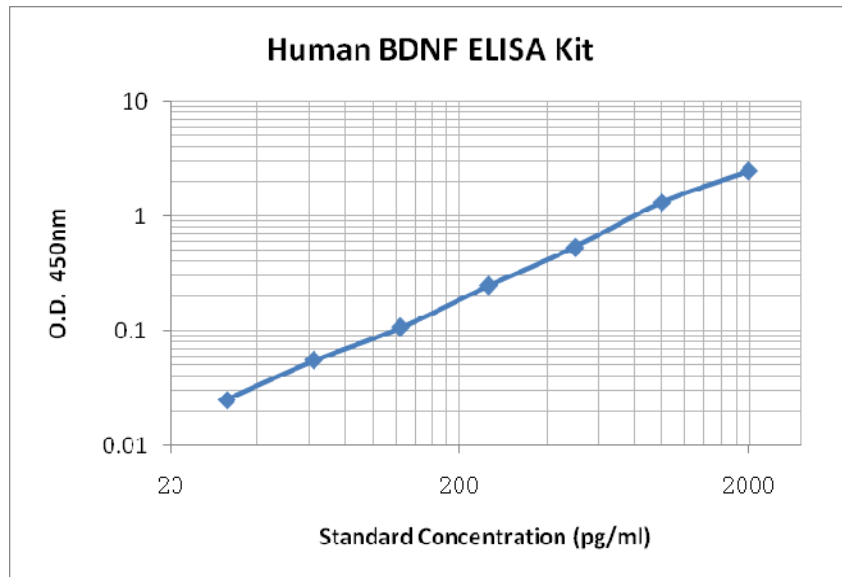
For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The **Human BDNF** concentration of the samples can 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.

Typical data:

This standard curve was generated at Novatein biolab for demonstration purpose only. A standard curve must be run with each assay.

Conc (pg/ml)	0	31.2	62.5	125	250	500	1000	2000
O.D.(450nm)	0.019	0.025	0.055	0.107	0.246	0.531	1.323	2.485



Background:

BDNF, also known as Abreineurin, is a secreted protein belonging to the nerve growth factor. The full-length BDNF precursor is a 247 amino acid (aa) prepropeptide composed of an 18 aa signal peptide, a 110 aa prosequence, and a 119 aa mature segment. Reportedly, the N-terminus of the immature BDNF might be alternatively spliced, generating a longer pre-prosegment (pro-BDNF). The N-terminal pro-BDNF is removed by tPA and produce the 119 aa mature BDNF. BDNF is highly expressed in hippocampus, amygdala, cerebral cortex and cerebellum. It is also expressed in non-neuronal tissues such as heart, lung, skeletal muscle, testis, prostate and placenta. BDNF plays very important roles in a variety of biological processes of central and peripheral systems. During development, it promotes the survival and differentiation of selected neuronal populations. It is involved in axonal growth, pathfinding and in the modulation of dendritic growth and morphology. It regulates synaptic transmission and plasticity at adult synapses in many regions of the brain. BDNF binds to the tyrosine kinase receptor (TrKB) and neurotrophin receptor (NTR) to transduce and mediate the downstream signaling cascades.

BDNF has been implicated in various pathological conditions of neurologic and/or mental disorders such as depression, bipolar disorder, schizophrenia, obsessive-compulsive disorder, Alzheimer's disease, Huntington's disease, Rett syndrome, and dementia. synaptic function and long-term potentiation.

Manufactured and Distributed by:
Novatein Biosciences
310 W Cummings Park, MA, 01810, USA
Phone: (617) 238-1396
Fax: (617) 380-0053
Toll Free: (888) 856-2858
<http://www.novateinbio.com/>
Email: Info@novateinbio.com