User Manual

Rat/Human Brain Derived Neurotrophic Factor ELISA Kit

CYT306

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Not for use in diagnostic procedures. Not for human or animal consumption.

Product Overview

Brain Derived Neurotrophic Factor (BDNF) kit is a sandwich enzyme immunoassay (EIA), which measures BDNF. The kit will measure BDNF from human and rat. BDNF acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses. In the brain, it is active in the hippocampus, cortex, and basal forebrain—areas vital to learning, memory, and higher thinking. BDNF itself is important for long-term memory. BDNF was the second neurotrophic factor to be characterized after nerve growth factor (NGF).

Although the vast majority of neurons in the mammalian brain are formed prenatally, parts of the adult brain retain the ability to grow new neurons from neural stem cells in a process known as neurogenesis. Neurotrophins are chemicals that help to stimulate and control neurogenesis, BDNF being one of the most active. Mice born without the ability to make BDNF suffer developmental defects in the brain and sensory nervous system, and usually die soon after birth, suggesting that BDNF plays an important role in normal neural development.

Test Principle

With the BDNF assay system, mouse monoclonal antibodies generated against human BDNF are coated onto a microplate and are used to capture BDNF from a sample. BDNF specific, biotin conjugated, mouse monoclonal antibodies detect the captured BDNF. After addition of streptavidin-enzyme, substrate and stop solution, the amount of BDNF is determined. The standard curve demonstrates a direct relationship between Optical Density (OD) and BDNF concentration: for example, the higher the OD the higher the BDNF concentration in the sample.

Application

BDNF kit is designed to measure the amount of BDNF in cell culture supernatants, tissue homogenates and biological fluid (serum, plasma, and serum-free) samples from human and rat. There are enough reagents included in this kit for two 96-well immuno-assay plates. Running duplicate wells for samples and standards is recommended.

Analytical Sensitivity and Detection Limits

Sensitivity:	15 pg/mL
Range of Detection:	15 pg/mL to 1000 pg/mL
Cross reactivity:	No significant cross reactivity with NGF, NT4/5 or NT3
Intra-assay Variation:	±3.7% (250 pg/mL)
Inter-assay Variation:	±8.5% (250 pg/mL)

Materials Provided

- BDNF ELISA Plate, 2 each (60238) - 96-well immunoplates pre-coated with Mouse anti-Human BDNF Monoclonal Antibody, sealed in foil pouches, 2 each.
- Wash Buffer 10X (100 mL) (60245) - one bottle of 10X buffer concentrate.
- Standard/Sample Diluent (60mL). One bottle, ready to use (60240).
- BDNF Standard (Recombinant Human), 2 each (60237) - Two vials, lyophilized.
- Biotinylated Mouse anti-Human BDNF Monoclonal Antibody (25 µL) (60583) - One vial.
- Streptavidin-Enzyme conjugate (50 µL) (60582) - one vial of HRP conjugated Streptavidin.
- TMB/E Solution (2 x 10 mL) (60096) - two bottles of a ready to use solution of 3,3',5,5'- Tetramethylbenzidine in a proprietary buffer with enhancer.
- Stop Solution (22 mL) (60260) - one bottle of an HCl solution.

Materials Required (Not supplied)

- Multi-channel or repeating pipettes
- Plate shaker (optional)
- Pipettors & tips capable of accurately measuring 10-1000 µL
- Graduated serological pipettes
- 96-well microplate Reader with 450 nm filter
- Graph paper for manual plotting of data
- Polystyrene test tubes for standard and sample dilutions
- Mechanical vortex
- One 1 L or 2 L container

Warnings and Precautions

Please refer to the product labels and the SDS available on the website for the complete hazard and precautionary statements for the components. The instructions provided have been designed to optimize the kit's performance. Deviation from the instructions may result in suboptimal performance of the kit and the failure to produce accurate data.

Storage and Stability

Maintain the unopened kit at 2-8 °C until expiration date indicated on the label. After opening the kit maintain the mouse anti-BDNF Monoclonal Antibody Coated Plate, Biotin Conjugated Mouse anti-BDNF Monoclonal Antibody, Wash Buffer, Sample Diluent, Streptavidin-Enzyme Conjugate, TMB Solution and Stop Solution at 2-8 °C until expiration date indicated on the label. Maintain the BDNF Standard, at 2-8 °C for up to 30 days after reconstitution.

Technical Hints

Manual Plate Washing: Vigorous washing and complete removal of all liquid by aspiration at the end of each washing step is very important to obtain low background values.

Recommended Method for Plate Washing:

1. Remove existing fluid from each well by flicking the plate over a sink. Subsequently blot the plate on clean paper towels.
2. Forcefully pipette 250 µL of diluted Wash Buffer into each well with a multi-channel pipette.
3. Remove fluid from each well by flicking the plate over a sink. Subsequently blot the plate on clean paper towels.
4. Repeat washing and flicking 4 times.

Preparation of Reagents

Biotinylated Mouse anti-Human BDNF Monoclonal Antibody

Immediately before use dilute the biotinylated antibody 1:1000 with Sample Diluent. Do not store diluted solutions.

Streptavidin-Enzyme Conjugate

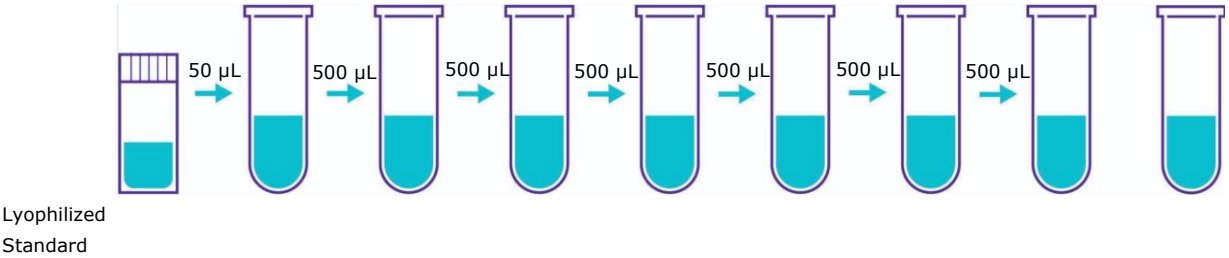
Immediately before use dilute the HRP conjugate 1:1000 with Sample Diluent. Do not store diluted solutions.

BDNF Standard

Note: When opening lyophilized Standard, remove rubber stopper gently as the lyophilizate may have become dislodged during shipping.

Reconstitute the standard vial with the volume of Sample Diluent indicated on the label to give a relative BDNF concentration of 20,000 pg/mL. This stock material is then used to generate a standard curve. Use the Sample Diluent to make the dilutions. A suggested dilution scheme is as follows:

1. Label 7 test tubes 1-7 and 1 test tube "0 dose". Add 950 µL of the Sample Diluent to Standard tube 1. Add 500 µL of the Sample Diluent to Standard tubes 2-7 and the "0 dose".
2. Add 50 µL of the stock Standard solution to tube 1 and vortex. This is Standard tube 1 with a concentration of 1000 pg/mL.
3. Standards 2-7 are then prepared by performing a 1:2 dilution of the preceding standard. Refer to Figure. 1. For example, to make Standard 2, remove 500 µL of Standard 1 and add it to tube 2 and vortex and so on. Do not add any BDNF Standard to the "0 Dose" Standard tube.



Standard Number	1	2	3	4	5	6	7	0 Dose
Initial Volume (µL)	950	500	500	500	500	500	500	500
Concentration (pg/mL)	1000	500	250	125	62.5	31.25	15.63	0.0

Figure 1: Serial Dilution of BDNF Standard

Note: The BDNF Standard curve can be set up with a different serial dilution scheme by making appropriate adjustments to the dilution pattern.

4. Add the entire contents of the 10X Wash Buffer concentrate to appropriate container, QS to 1 L with deionized water. Stir to homogeneity.

Preparation of Samples

It is recommended that you test each sample in duplicate. The samples should be diluted with Sample/Standard Diluent 1:2 and further diluted by a two-fold serial dilution and run down a column of the plate. Alternatively, you can screen samples at a single concentration (in triplicate) and re-assay all positive samples to determine the exact BDNF concentration.

Tissue samples should be rapidly excised, weighed and snap frozen in liquid nitrogen prior to storage at −70 °C. Within two weeks of freezing, tissue samples should be homogenized in ice cold homogenization buffer consisting of 100 mM tris/HCl (pH 7), containing 2% bovine serum albumin (BSA), 1 M NaCl, 4 mM EDTA.Na_2, 2% Triton™ X-100, 0.1% sodium azide and the protease inhibitors 5 µg/mL aprotinin, 0.5 µg/mL antipain, 157 µg/mL benzamidine, 0.1 µg/mL pepstatin A and 17 µg/mL phenylmethyl-sulphonyl fluoride.

Homogenates should be prepared in approximately 20 to 100 volumes of the homogenization buffer to tissue wet weight, but the most appropriate ratio needs to be determined by the user for each tissue. The homogenates are centrifuged at 14,000 x *g* for 30 minutes. The resulting supernatants should be used for the BDNF assay.

Assay Instructions

1. Place the desired number of Brain Derived Neurotrophic Factor strips in the strip well plate holder.
2. Add 100 µL of Standards 0 through 7 or samples to wells. It is recommended that standards and samples be run in duplicate.

   Note: A standard curve must be run at each setting.
3. Seal the plate with a plate sealer. Incubate the plate at 2-8 °C overnight (on a shaker if possible).
4. **Important Wash Step:**

   Gently remove the plate sealer and wash the plate at least 4 times. A thorough washing of the plate is extremely important to reduce background. We recommend using a multi-channel pipette to fill each well with 250 µL of diluted Wash Buffer. Fluid removal from the wells is best accomplished by inverting the plate over a sink and flicking the fluid out of the wells and then blotting the plate on clean paper towels. Using the multichannel pipette add 250 µL of Wash Buffer to each well; flick and blot the plate.

   Repeat this procedure for a total of 4 times.

   For users of automatic plate washers: It is important to ensure that the wash apparatus is properly maintained and operating correctly. Tubing and tips can easily become clogged, leading to incomplete washing and inadequate aspiration of wells. The result may be poor precision and an unsuitable standard curve. For best results, we recommend at least 4 wash cycles.
5. Add 100 µL of the diluted biotinylated mouse anti-BDNF monoclonal antibody (see reagent preparation section) to each well. Cover the plate and incubate at room temperature for 2-3 hours (on shaker if possible). Wash as described in Step 4 (Important Wash Step).
6. Add 100 µL of the diluted streptavidin-HRP conjugate solution (see reagent preparation section) to each well. Cover the plate and incubate at room temperature for 1 hour (on shaker if possible). Wash as described in Step 4 (Important Wash Step).
7. Warm TMB/E to room temperature. Add 100 µL of TMB/E Substrate to each well. Incubate at room temperature for 15 minutes. (The 1000 pg/mL standard should achieve a deep blue color). Stop the reaction by adding 100 µL of Stop Solution to each well. The blue color will change to yellow. Immediately read the plate at 450 nm (color will fade over time).

   Caution: Bubbles in the wells will cause inaccurate readings. Ensure that all bubbles are removed prior to taking the absorbance reading.

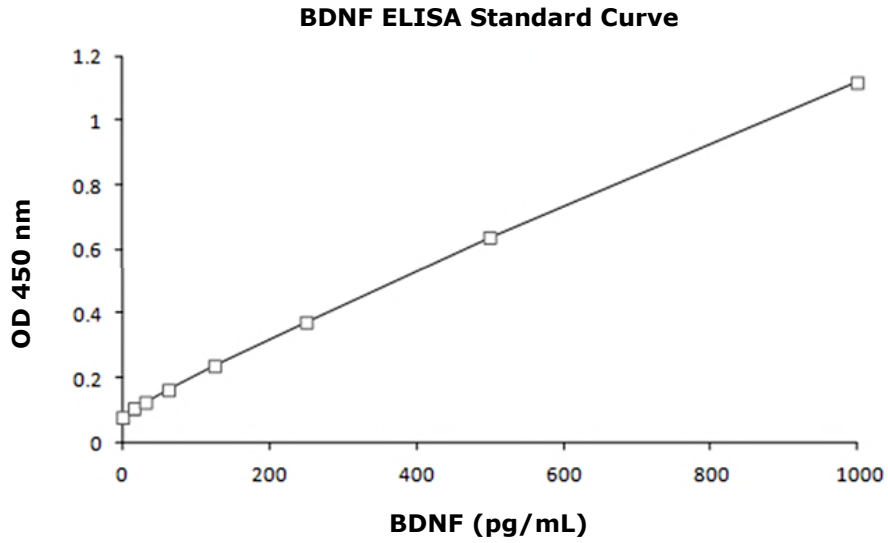
Calculation of Results

Manual Plotting

Plot the standard curve on graph paper. Known concentrations of BDNF are plotted on the X-axis and the corresponding OD on the Y-axis. The standard curve should result in a graph that shows a direct relationship between BDNF concentrations and the corresponding ODs (absorbances). In other words, the greater the concentration of BDNF in the sample, the higher the OD. The concentration of BDNF in unknown samples may be determined by plotting the sample OD on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point intersects the X-axis at the concentration of BDNF in the unknown sample.

Plate Reader/PC Interface

An alternative approach is to enter the data into a computer program with curve fitting software. A good fit can be obtained with a linear regression analysis. Some data points at the top or bottom of the range tested may need to be dropped to get a good fit. Currently existing spreadsheet software can perform such plotting.



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