Human BDNF ELISA Kit

Cat No. ELI-M-005-96
Size: 96 tests

MOLEQULE-ON®

Components	Quantity
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 Plate
Standard - lyophilized, 1000 pg/ml upon reconstitution	3 Vials
Concentrated Biotin-Conjugated antibody	1 Vial
Concentrated Streptavidin-HRP solution	1 Vial
Reagent Diluent – 21ml/vial	1 Bottle
Assay Buffer Concentrate (20X) – 20ml/vial	1 Bottle
20X PBS Buffer – 20ml/vial	1 Bottle
Substrate Solution – 10.5 ml/vial	1 Bottle
Stop Solution – 5.5 ml/vial	1 Bottle
Plate Cover Seals	2 pieces

Reagent Preparations

1. Temperature returning - Bring all kit components and specimen to room temperature (20-25℃) before use.

2. Working Solution of Assay Buffer: Dilute 20ml of Assay Buffer Concentrate with 380mL of deionized or distilled water to prepare 400ml of 1X Assay Buffer. If crystals have formed in the concentrate Assay Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. Working Solution of PBS: Dilute 20mL of 20X PBS with 380ml of deionized or distilled water to prepare 400ml of 1X PBS.

3. Standard/Specimen - Reconstitute the Standard with 500µl of 1X Assay Buffer. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µl of 1X Assay Buffer into six tubes and marked them as 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml and 15pg/ml. Make a serial dilution by pipetting 250µl standard into first tube (500pg/ml) and then pipette 250µl from first dilution (500pg/ml) to second tube (250pg/ml) and subsequently add 250µl from each tube to next tube to make serial dilution of mentioned concentration. Mix each tube thoroughly and change pipette tips between each transfer. The 1000pg/ml standard serves as the high standard. The 1X Assay Buffer serves as the zero standard (0pg/ml). Serially diluted standard shall not be reused.

4. Working solution of Biotin-Conjugate anti-human BDNF antibody: Centrifuge for 1 min at 6000 rpm to bring down the material prior to open the vial. Add 200µl of sterile 1X PBS to the vial and vortex the reconstituted antibody for 30 seconds. Add 20µl of reconstituted Biotin-conjugate solution to 980µl of Reagent Diluent to prepare working solution of Biotin-Conjugate anti-human BDNF antibody. If the entire 96-well plate is used, take the entire 200µl of antibody to 10ml of Reagent Diluent and mix thoroughly prior to the assay. If the partial antibody is used store the rest at -20°C until use.
***The working solution should be used within one day after dilution.**

5. Working solution of Streptavidin-HRP: Centrifuge for 1 min at 6000 rpm to bring down the material prior to open the vial. The vial contains 50µL HRP Conjugate sufficient for one 96-well plate. (If the volume is less than 50µL, add sterile 1X PBS to reach 50µL and vortex for 10 seconds). Add 5µl of Streptavidin-HRP Conjugate to 995µl of Reagent Diluent to prepare working solution of streptavidin-HRP. If the entire 96-well plate is used, add 50µL of HRP Conjugate to 10ml of Reagent Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 3 months. DO NOT FREEZE.
***The working solution should be used within one day after dilution.**

Storage

All components of Human BDNF ELISA Kit should be store at 2-8°C except reconstituted standard that should be store at -20℃.

Description

BDNF is made in the endoplasmic reticulum and secreted from dense-core vesicles. It binds carboxypeptidase E (CPE), and the disruption of this binding has been proposed to cause the loss of sorting of BDNF into dense-core vesicles. The phenotype for BDNF knockout mice can be severe, including postnatal lethality. Other traits include sensory neuron losses that affect coordination, balance, hearing, taste, and breathing. Knockout mice also exhibit cerebellar abnormalities and an increase in the number of sympathetic neurons. Various studies have shown possible links between BDNF and conditions such as depression, bipolar disorder, schizophrenia, obsessive-compulsive disorder, Alzheimer's disease, Huntington's disease, Rett syndrome, and dementia, as well as anorexia nervosa and bulimia nervosa.

Principle

This assay is to determine BDNF density in Human samples. This is a shorter ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for Human BDNF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BDNF present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Human BDNF is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of BDNF bound in the initial step. The color development is stopped, and the intensity of the color is measured.

Sample Collection And Storage

1. Cell Culture Supernates - Centrifuge cell culture media at 1000 x g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
2. Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000g (or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
3. Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g (or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

Procedure

Prepare all reagents and working standards as directed in the previous section.
1. Add 100μL of sample (such as plasma or serum) or standard per well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1X Assay Buffer. Wash by filling each well with 300μl of 1X Assay Buffer using a multi-channel pipette, semi-automated or automated washer. Complete removal of liquid at each step is essential for good performance. Repeat the washing step twice. After the last wash, remove any remaining Assay Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100μL of the working dilution of Biotin-Conjugate anti-human BDNF antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100μL of the working dilution of HRP Conjugate to each well. Cover the plate and incubate for 20 minutes at room temperature. Keep the plate in dark condition.
6. Repeat the aspiration/wash as in step 2.
7. Add 100μL of Substrate Solution to each well. Incubate for 5-20 minutes (depending on signal) at room temperature. Keep the plate in dark condition.
8. Add 50μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Technical Note

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

Calculation of Results

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the BDNF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Sensitivity, Specificity And Repeatability

1. **REPEATABILITY**: The coefficient of variation of both intra-assay and inter-assay was less than 10%.
2. **SENSITIVITY**: The minimum detectable dose was 3pg/ml.
3. **SPECIFICITY**: This assay recognizes both natural and recombinant human BDNF.

References

1. Robinson RC, Radziejewski C, Stuart DI, Jones EY (April 1995). Biochemistry 34 (13): 4139–46.
2. Jones KR, Reichardt LF (October 1990). Proc. Natl. Acad. Sci. U.S.A. 87 (20): 8060–4.
3. Acheson A, Conover JC, Fandl JP, et al. (March 1995). Nature 374 (6521): 450–3.
4. Yamada K, Nabeshima T (April 2003). J. Pharmacol. Sci. 91 (4): 267–70.
5. Bekinschtein P, Cammarota M, et al. (2008). Proc. Natl. Acad. Sci. U.S.A. 105 (7): 2711–6.
6. Dwivedi Y (2009). Neuropsychiatr Dis Treat 5: 433–49.
7. Brunoni AR, Lopes M, Fregni F (December 2008). Int. J. Neuropsychopharmacol. 11 (8): 1169–80.
8. Iria Grande, Gabriel Rodrigo Fries, et al. Psychiatry Investig. 2010 December; 7(4): 243–250.