biosensis® BDNF Rapid™ ELISA Kit: Human, Mouse, Rat (1 Plate)
Catalogue Number: BEK-2211-1P

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1. Introduction

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.

The Biosensis BDNF Rapid™ enzyme-linked immunosorbent assay (ELISA) Kit is a sandwich ELISA that allows the quantification of 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). The amino acid sequence of BDNF is identical for human, mouse, rat and a number of other species. This kit therefore is suitable to measure BDNF in all these species and uses the same antibodies and antigen.

The antibodies used in this ELISA kit bind epitopes within the mature domain of the protein and therefore recognize the pro-, mature and truncated form of BDNF (Figure 1).

The purpose of this kit is the in vitro quantitative determination of BDNF in samples such as serum, plasma (EDTA and citrate), cell culture supernatants and brain extracts. For quantification of BDNF in CSF samples please contact us sales@biosensis.com. This kit has not been tested for other applications.

NOTE: For research use only. Not for diagnostic and clinical purposes.

2. Materials Provided and Storage Conditions

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF antibody coated 96 well microplate</td>
<td>1 plate</td>
</tr>
<tr>
<td>Assay diluent*1 (1x)</td>
<td>2 x 25 mL</td>
</tr>
<tr>
<td>Recombinant BDNF standard</td>
<td>2 x 1000 pg</td>
</tr>
<tr>
<td>BDNF detection antibody (100x)</td>
<td>1 x 110 µL</td>
</tr>
<tr>
<td>Streptavidin-HRP (100x)</td>
<td>1 x 110 µL</td>
</tr>
<tr>
<td>Wash buffer (10x)</td>
<td>1 x 33 mL</td>
</tr>
<tr>
<td>TMB substrate (1x)</td>
<td>1 x 11 mL</td>
</tr>
<tr>
<td>TMB stop solution (1x)</td>
<td>1 x 11 mL</td>
</tr>
<tr>
<td>Plate sealer</td>
<td>1</td>
</tr>
</tbody>
</table>

*1 The assay diluent provided in this kit is suitable for measuring BDNF in serum, plasma and cell culture supernatants. For brain extracts, please see information provided in Section 5 and Appendix A.

- The ELISA kit may be stored unopened for up to 12 months at 2-8°C from the date of shipment
- The detection antibody may be aliquoted and stored at -20°C for extended storage
- Do not freeze the streptavidin-HRP conjugate!
- The reconstituted BDNF standard may be stored in aliquots at -20°C for 1 week.

3. Equipment Required but Not Supplied

- Single- and multi-channel pipettes capable of delivering 10-1000 µL volumes
- Plastic and glass ware for sample collection, sample preparation and buffer preparation
- Plate shaker
- Microplate reader capable of reading absorbance at 450 nm
4. Before You Start….
   - Bring the microplate and all reagents and solutions to room temperature before starting the assay
   - Remove only the required amount of TMB substrate from the fridge to prevent multiple warm-ups and cool downs; keep the TMB substrate in the dark
   - Remove the number of strips required and return unused strips to the pack and reseal
   - Centrifuge all kit components to collect reagents to the bottom of tubes before use
   - The stop solution provided with this kit is an acid; wear protective equipment when handling

5. Sample Preparation
   For unknown concentrations of BDNF in samples, it is imperative 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).

   **Cell Culture Supernatants**
   - Remove particulates by centrifugation (10,000 x g for 5 minutes)
   - Analyze immediately or store at -20°C to -80°C in aliquots to prevent multiple freeze thaw cycles
   - If required, dilute serum samples with the assay diluent provided in order to measure BDNF concentrations

   **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 the assay diluent provided in order to measure BDNF concentrations
   - For human serum samples, we suggest a sample dilution range of 1/50 – 1/400

   **Note:** Acid treatment for serum samples is not required.

   **Plasma**
   - Collect plasma using EDTA or citrate as anticoagulant
   - 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 with the assay diluent provided in order to measure BDNF concentrations

   **Note:** Acid treatment for plasma samples is not required.

6. Preparation of BDNF Standard
   • Reconstitute the lyophilized antigen standard with 1 mL of the same assay 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 assay 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 dilution 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, you may want to follow 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 assay 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

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5. Repeat steps 3 and 4 for each consecutive concentration until the last tube “7.8 pg/mL” is prepared and mixed well

<table>
<thead>
<tr>
<th>BDNF (pg/mL)</th>
<th>500</th>
<th>250</th>
<th>125</th>
<th>62.5</th>
<th>31.3</th>
<th>15.6</th>
<th>7.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final volume in vial (μL)</td>
<td>600</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>800</td>
</tr>
</tbody>
</table>

7. **Other Reagents and Buffer Preparation**

- Detection antibody (100x) and streptavidin-HRP conjugate (100x): dilute one hundred-fold with the assay diluent used to prepare samples and BDNF standard; prepare enough volume to add 100 μL per well.
- Wash buffer (10x): dilute ten-fold with ultrapure water; each 8-well strip requires about 25 mL of 1x wash buffer per assay

8. **Assay 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 (assay 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 (eg., with plate sealer or parafilm) and incubate the plate on a shaker (140 rpm) for 30 minutes
4. Discard the solution inside the wells and perform 5 washes with 1x wash buffer (200 μL per well). See the technical hints section for a detailed description of the washing procedure
5. Add 100 μL of the detection antibody (1x) into each well
6. Seal the plate (eg., with plate sealer or parafilm) and incubate the plate on a shaker (140 rpm) for 15 minutes
7. Discard the solution inside the wells and wash as described in step 4
8. Add 100 μL of the 1x streptavidin-HRP conjugate into each well
9. Seal the plate (eg., with plate sealer or parafilm) and incubate the plate on a shaker (140 rpm) 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 4-15 minutes without shaking in the dark
12. Stop the reaction by adding 100 μL 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
13. See Appendix B for a troubleshooting guide when unexpected difficulties are encountered

9. **Technical Hints**

1. Do not perform dilutions within the well
2. At least duplicate measurements for each standard and sample dilution is recommended
3. Dilute samples to a BDNF concentration that falls within the range of the standard curve. Do not extrapolate absorbance readings
4. Avoid touching the inside surface of the wells with the pipette tip
5. Proper emptying and washing the plate is crucial for low background and 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 5 times
Protocol for Quantification of Human, Mouse and Rat BDNF

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1. 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.
2. Add TMB and the stop solution to the wells in the same order.
3. Ensure that all bubbles are removed and that the bottom of the plate is optically clean prior to taking the absorbance reading.
4. Stopping the TMB reaction after 5 minutes is usually sufficient to obtain a very sensitive standard curve. However, TMB incubation times may vary based on the accuracy of diluting the ELISA kit reagents and differences in incubation time and temperature. We use a plate shaker set to 140 rpm and perform all incubations at room temperature (20-25°C, 70-75°F) in our laboratories. Importantly, we read our plates on a microplate reader capable of measuring up to an optical density (OD) of 4. You may need to adjust the TMB incubation time if your microplate reader has a more limited range.

10. 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 OD_{450} of the samples as well.
4. Use appropriate software to reduce the data and generate a four parameter logistic (4-PL) curve-fit.
5. Perform a regression analysis to calculate the concentration of BDNF in the samples. Multiply the result by the sample dilution factor.

Manual Plate Reading
The relative OD_{450} = (the OD_{450} of each well) – (the OD_{450} of Zero well).

1. The **standard curve** can be plotted as the relative OD_{450} of each BDNF standard solution (Y-axis) vs. the respective known concentration of the BDNF standard solution (X-axis).
2. The standard curve should result in a graph that shows a direct relationship between target protein concentrations and the corresponding OD_{450}. The greater the concentration of target protein in the solution, the higher the OD_{450}.

3. **Determine concentration of target protein in unknown sample.** The BDNF protein concentration of the samples can be interpolated from the standard curve. Draw 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. In the exemplary standard curve on the right, a sample with OD_{450} = 1 reads as 80 pg/mL BDNF (red line).

4. If the samples measured were diluted, multiply the concentrations from interpolation (see step 3) with the dilution factor to obtain the actual BDNF concentration in the sample.

11. Typical Data

Standard Curve
Standard curves are provided for demonstration only. A standard curve has to be generated for each BDNF ELISA assay.

In addition, we recommend measuring common samples when performing multiple assays across several days in order to normalize the standard curve numbers between the various runs.

In the above example graph, BDNF standards were run in triplicates using the assay diluent provided in this kit. The TMB reaction was stopped after 5 minutes.
Typical optical densities and coefficient of variations are summarized in the following table:

<table>
<thead>
<tr>
<th>BDNF / [pg/mL]</th>
<th>OD 450 nm</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>3.068</td>
<td>0.052</td>
<td>0.030</td>
<td>0.003</td>
<td>1.68%</td>
</tr>
<tr>
<td>250</td>
<td>2.147</td>
<td>0.019</td>
<td>0.011</td>
<td>0.007</td>
<td>0.90%</td>
</tr>
<tr>
<td>125</td>
<td>1.507</td>
<td>0.013</td>
<td>0.007</td>
<td>0.004</td>
<td>0.85%</td>
</tr>
<tr>
<td>62.5</td>
<td>0.902</td>
<td>0.024</td>
<td>0.014</td>
<td>0.006</td>
<td>2.70%</td>
</tr>
<tr>
<td>31.3</td>
<td>0.546</td>
<td>0.010</td>
<td>0.006</td>
<td>0.004</td>
<td>1.91%</td>
</tr>
<tr>
<td>15.6</td>
<td>0.304</td>
<td>0.010</td>
<td>0.006</td>
<td>0.002</td>
<td>3.29%</td>
</tr>
<tr>
<td>7.8</td>
<td>0.183</td>
<td>0.003</td>
<td>0.002</td>
<td>0.001</td>
<td>1.66%</td>
</tr>
<tr>
<td>Blank</td>
<td>0.060</td>
<td>0.003</td>
<td>0.002</td>
<td>0.001</td>
<td>5.62%</td>
</tr>
</tbody>
</table>

SD: standard deviation; SEM: standard error of mean; CV: coefficient of variation

**Limit of Detection**

This BDNF ELISA kit detects down to 2 pg/mL BDNF (defined as 150% of blank value).

**Linearity of Dilutions**

Two human serum samples were each diluted 1/50 – 1/400 with the assay diluent provided in this kit and assayed in triplicates. A 4-PL regression analysis was carried out to determine mean BDNF levels in serum.

The BDNF concentrations in human serum varied between 95.8–103.6% of the mean BDNF concentration within the dilution range.

**Recovery**

Two human serum samples were each spiked with 3 different levels of BDNF (20, 50, 100 pg/mL) at a sample dilution of 1/100. No matrix interference was observed.

**Assay Precision**

Two human serum samples were assayed in triplicates on 3 different days.

<table>
<thead>
<tr>
<th>BDNF / [ng/mL]</th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Day 1</td>
<td>25.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Day 2</td>
<td>26.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Day 3</td>
<td>24.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

SD: standard deviation; SEM: standard error of mean; CV: coefficient of variation

**Specificity**

The antibodies used in this ELISA kit bind epitopes within the mature domain of the protein and therefore recognize the pro-, mature and truncated form of BDNF. They do not cross-react with nerve growth factor (NGF), neurotrophin-3 (NT-3) and NT-4/5.

**Reference**

Kolbeck R et al., Brain-derived neurotrophic factor levels in the nervous system of wild-type and neurotrophin gene mutant mice. J Neurochem. 1999 May;72(5):1930-8.

**Other Information**

We recommend the Biosensis proBDNF Rapid™ ELISA kit (Cat# BEK-2217-2P) to quantify proBDNF independent of mature BDNF (Figure 2).
Appendix A: Acid-Extraction of BDNF from Mammalian Brain Tissue

BDNF is bound to its receptors within many tissues, which prevents its detection in any ELISA. Acid extraction protocols have therefore been developed to release this bound BDNF, which not only release the bound BDNF, but also precipitates the receptors. We recommend the following sample preparation protocol for measuring total BDNF concentrations in acid-treated tissue samples. This protocol is based on Kolbeck et al. (1999) as published by researchers from Professor Y.-A. Barde's laboratory. Our ELISA assay has been validated using this protocol.

Extraction Buffer
50 mmol/L sodium acetate, 1 mol/L NaCl, 0.1% Triton X100, add acetic acid until pH 4.0 is reached. Before use add one “Complete” or “Complete Mini” protease inhibitors cocktail tablet (Roche, cat. no. 11697498001 or 11836153001), to be used as recommended by the manufacturer.

Incubation/Neutralization Buffer
0.1 mol/L phosphate buffer (0.1 mol/L KH₂PO₄ + 0.1 mol/L Na₂HPO₄), pH 7.6.

Brain Tissue Assay Diluent
Mix extraction buffer and incubation/neutralization buffer at a ratio of 1 to 3 (e.g., 1 mL of extraction buffer and 3 mL of incubation/neutralization buffer).

Protocol
1. Dissect brain structures, weigh tissue fragments and then freeze them rapidly in liquid nitrogen
2. For long term storage transfer the frozen tissue samples to -80°C
3. Re-suspend brain tissues in approximately 10 weight/volume-ratio of extraction buffer (for example, 100 µL extraction buffer for 10 mg tissue)
4. Sonicate the suspension to homogeneity on ice with a probe sonicator (e.g. Misonix Ultrasonic Liquid Processor)
5. Sonicate in short bursts (5-7 sec) to avoid excessive sample heating
6. Keep homogenates on ice for 30 min, then repeat sonication as well as incubation on ice
7. Centrifuge homogenates for 30 minutes at 100,000 x g and 4°C, then transfer clear supernatants into clean tubes and discard pellets
8. Measure total protein concentration (e.g., BCA or Bradford protein assay)
9. These supernatants may be stored at -80°C and must be centrifuged again for 30 min at 20,000 x g and 4°C immediately after thawing and before being loaded into the wells of an ELISA plate
10. Reconstitute the supplied BDNF standard with 1 mL of Brain Tissue Assay Diluent; perform a 1:2 serial dilution as outlined in Section 6 (“Preparation of BDNF Standard”).
11. Prepare a sample dilution with 1 part tissue extract and 3 parts of Incubation/Neutralization Buffer (e.g., 50 µL tissue extract and 150 µL Incubation/Neutralization buffer); this equals a sample dilution factor of 4 which needs to be considered when calculating BDNF concentrations in samples
12. If the BDNF concentration in the sample is out of the assay range (7.8-500 pg/mL), dilute the solution prepared in step 11 further with Brain Tissue Assay Diluent
13. Results can then be reported as ng BDNF/mg total soluble protein or g wet weight if tissue sample is large enough

Note: Biosensis recommends evaluating the recovery of BDNF when preparing tissue extracts. In order to conduct a recovery experiment, known amounts of BDNF standard are added to an aliquot of the brain tissue homogenates and assayed.
Appendix B: Troubleshooting Guide

This BDNF ELISA kit has been developed to deliver reproducible results when following the provided assay protocol. Please refer to the troubleshooting guide below when unexpected difficulties are encountered. If you require further assistance, talk to a scientist at Biosensis (biospeak@biosensis.com).

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High background (blank OD &gt; 0.30)</strong></td>
<td>Insufficient washing</td>
<td>Make sure to perform 5 washes after each incubation step with sample, detection antibody and streptavidin-HRP conjugate. Follow the recommended manual washing procedure for optimal washing performance</td>
</tr>
<tr>
<td></td>
<td>Excessive concentration of detection antibody and/or HRP-conjugate</td>
<td>Re-examine the calculations performed to dilute the concentrated stock solutions. Verify that the pipettes dispense the correct volume.</td>
</tr>
<tr>
<td></td>
<td>Increased incubation time and temperature</td>
<td>Please follow incubation times as stated in the protocol and perform incubations at room temperature</td>
</tr>
<tr>
<td></td>
<td>Contamination</td>
<td>Ensure that all plastic- and glassware used is clean. Do not use the same pipette tip to transfer samples and blank solution into the wells</td>
</tr>
<tr>
<td><strong>Low absorbance readings</strong></td>
<td>Concentration of BDNF in the sample is lower than the detection limit of this assay</td>
<td>Confirm that the assay protocol was carried out correctly by either spiking a known amount of BDNF into your sample or use a sample with known concentration of BDNF as positive control</td>
</tr>
<tr>
<td></td>
<td>Insufficient antibody or insufficient HRP-conjugate</td>
<td>Re-examine the calculations performed to dilute the concentrated stock solutions. Verify that the pipettes dispense the correct volume</td>
</tr>
<tr>
<td></td>
<td>Reagents expired</td>
<td>Ensure correct storage of the kit and do not use the kit beyond its expiry date</td>
</tr>
<tr>
<td><strong>Low absorbance readings</strong></td>
<td>Decreased incubation times and temperature</td>
<td>Please follow incubation times as stated in the protocol and warm reagents to room temperature before use</td>
</tr>
<tr>
<td></td>
<td>Microplate wells dried out</td>
<td>Do not leave microplate wells without solution for an extended time</td>
</tr>
<tr>
<td></td>
<td>Wash buffer not diluted</td>
<td>Ensure that the wash buffer is diluted with ultrapure water to a 1x working solution</td>
</tr>
<tr>
<td></td>
<td>Stop solution not added</td>
<td>Add the stop solution to obtain a yellow reaction product that can be measured at 450 nm</td>
</tr>
<tr>
<td><strong>Standard OD values above plate reader limit</strong></td>
<td>Excessive incubation with TMB substrate solution</td>
<td>Reduce incubation time by stopping the reaction at an earlier time-point</td>
</tr>
<tr>
<td><strong>Sample OD values above standard curve range</strong></td>
<td>BDNF concentration in sample is too high</td>
<td>Perform a serial dilution of your sample to obtain a reading that falls within the standard curve</td>
</tr>
<tr>
<td><strong>High coefficient of variations (CV)</strong></td>
<td>TMB and stopping solution not added consistently</td>
<td>Add TMB and stopping solution to the wells in the same order and speed</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Ensure pipettes are working correctly and are calibrated; review your pipetting technique; add standards and samples to the wells using a single-channel rather than a multi-channel pipette</td>
</tr>
<tr>
<td></td>
<td>Insufficient mixing of reagents</td>
<td>Briefly vortex to mix solutions before pipetting into the wells</td>
</tr>
<tr>
<td></td>
<td>Bottom of the plate is dirty</td>
<td>Clean the bottom of the plate before reading the plate</td>
</tr>
</tbody>
</table>