REFERENCES





# Rat BDNF Immunoassay

Catalog Number: SEKR-0076

For the quantitative determination of rat BDNF concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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**LINEARITY:**To assess the linearity of the assay, three samples were spiked with high concentrations of Rat BDNF in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

| Dilution ratio | Recovery(%) | Citrate plasma | Cell culture supernatants |
|---|---|---|---|
| 1:2 | Average% of Expected | 97 | 106 |
| | Range(%) | 92—103 | 97—115 |
| 1:4 | Average% of Expected | 101 | 105 |
| | Range(%) | 96—110 | 96—114 |
| 1:8 | Average% of Expected | 103 | 110 |
| | Range(%) | 95-111 | 103-117 |
| 1:16 | Average% of Expected | 108 | 116 |
| | Range(%) | 102-114 | 109-123 |

## Performance Characteristics

**SENSITIVITY:** The minimum detectable dose was 2pg/mL.
**SPECIFICITY:** This assay recognizes both natural and recombinant rat BDNF. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

| Recombinant rat | Recombinant mouse | Other proteins |
|---|---|---|
| β-NGF | BDNF | |
| | β-NGF | |
| | NGF R | |
| | NT-4 | |
| | TrkB | |
| | TrkC | |
| | | |
| | | |
| | | |
| | | |

**REPEATABILITY:** The coefficient of variation of both intra-assay and inter-assay were less than 10%.
**RECOVERY:** The recovery of Rat BDNF spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of Rat BDNF in two matrices

| Sample Type | Average % of Expected Range(%) | Range(%) |
|---|---|---|
| Citrate plasma | 91 | 85—95 |
| Cell culture supernatants | 107 | 98—115 |



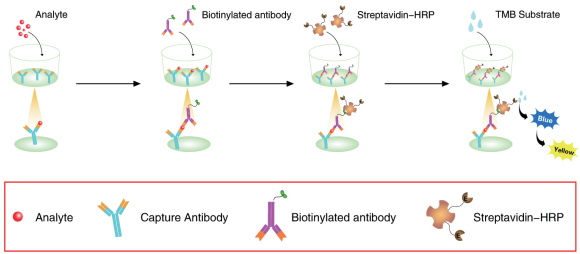


## BACKGROUND

BDNF (Brain-Derived Neurotrophic Factor) is a secreted protein that regulates many aspects of neuronal development and function in the nervous system. It is produced as proBDNF in response to neuronal activity or inflammatory stimulation and is then cleaved before associating into a homodimer. It is expressed throughout the nervous system as well as by fibroblasts, megakaryocytes/platelets, and smooth muscle cells. BDNF regulates neural stem cell survival and differentiation, axon/dendrite differentiation, synapse formation and maturation, and refinement of developing circuits. It is also a key regulator of synaptic plasticity and late-phase long-term potentiation. BDNF signals through the TrkB and NGF R/TNFRSF16 receptors. Secreted proBDNF can bind to NGF R/TNFRSF16 and induce long-term depression and cell apoptosis.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for BDNF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BDNFpresent is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for BDNF is added to detect the captured BDNF protein in sample. For signal development, horseradish peroxidase (HRP)-conjugatedStreptavidinis added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.




## TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

## PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

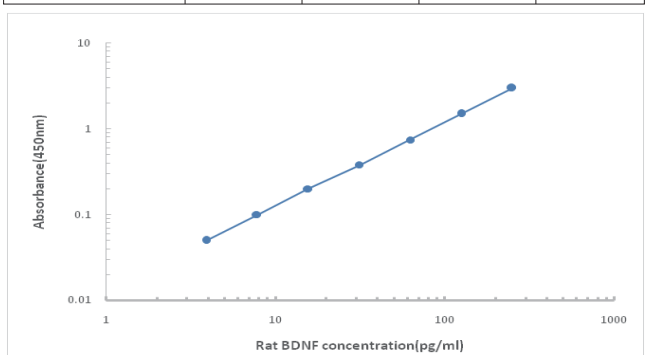




determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the Rat BDNF ELISA

| Standared(pg/ml) | OD. | OD. | Average | Corrected |
|---|---|---|---|---|
| 0 | 0.048 | 0.048 | 0.048 | - |
| 3.91 | 0.165 | 0.172 | 0.168 | 0.120 |
| 7.8 | 0.240 | 0.250 | 0.245 | 0.197 |
| 16 | 0.357 | 0.371 | 0.364 | 0.316 |
| 31.25 | 0.567 | 0.590 | 0.578 | 0.530 |
| 62.5 | 0.921 | 0.958 | 0.939 | 0.891 |
| 125 | 1.499 | 1.560 | 1.529 | 1.481 |
| 250 | 2.442 | 2.542 | 2.492 | 2.444 |




Representative standard curve for Rat BDNF ELISA.

## ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, incubate 120 minutes,at room temperature (25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-rat Rat BDNF antibody to each well, incubate 60 minutes,at room temperature (25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, incubate 30 minutes,at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 10-30 minutes,at room temperature (25±2℃).Protect from light.

⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

## 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 Rat BDNF concentrations versus the log of the O.D. and the best fit line can be





## KIT COMPONENTS& STORAGE CONDITIONS

| PART | SIZE | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|
| Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips) | 1 plate | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃** |
| Standard - lyophilized,1000 pg/ml upon reconstitution | 2 vials | Store at 2-8°C **for six months |
| Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial | 1 vial | Store at 2-8°C **for six months |
| Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial | 1 vial | Store at 2-8°C **for six months |
| Standard /sample Diluent - 16 ml/vial | 1 bottle | Store at 2-8°C **for six months |
| Biotin-Conjugate antibody Diluent - 16 ml/vial | 1 bottle | Store at 2-8°C **for six months |
| Streptavidin-HRP Diluent - 16 ml/vial | 1 bottle | Store at 2-8°C **for six months |
| Wash Buffer Concentrate (20x) - 30 ml/vial | 1 bottle | Store at 2-8°C **for six months |
| Substrate Solution - 12 ml/vial | 1 bottle | Store at 2-8°C **for six months |
| Stop Solution - 12 ml/vial | 1 bottle | Store at 2-8°C **for six months |
| Plate Cover Seals | 4 pieces | |

**Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

## SPECIMEN COLLECTION & STORAGE

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

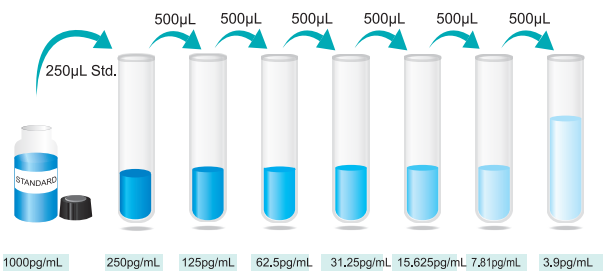
## REAGENTS PREPARATION

1. **Temperature returning -** Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer -** Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard\Specimen -** Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution





of 1000pg/mL.Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 750uL of Standard/Sample Diluent into the 250 pg/mL tube, and add 250uL stock solution of 1000 pg/mL into it to get the high standard of 250 pg/mL. Pipette 500uL of Standard/Sample Diluent into 125pg/ml tube and the remaining tubes. Use the stock solution of 250pg/mL to produce a 2-folddilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 250pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).




Preparation of BDNF standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-rat Rat** BDNF **antibody**:Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**