

# BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) (HUMAN, MOUSE, RAT) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF  
HUMAN, MOUSE AND RAT BDNF  
CONCENTRATIONS IN *CELL CULTURE SUPERNATES*,  
SERUM



FOR RESEARCH USE ONLY. NOT FOR USE IN  
DIAGNOSTIC PROCEDURES.

## PURCHASE INFORMATION:

ELISA NAME	BDNF (HUMAN, MOUSE, RAT) ELISA
Catalog No.	SK00752-01
Lot No.	
Formulation	96 T
Standard range	23-1500 pg/ml
Sensitivity	5-8 pg/ml
Sample Volume	100 µl
Sample Type	Serum, Cell Culture Supernates
Dilution Factor	<i>40 (Optimal dilutions should be determined by each laboratory for each application)</i>
Specificity	BDNF mature form at 100% Active human Pro BDNF derived from human cells at less 1%
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8 °C

## ORDER CONTACT:

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## INTRODUCTION

This BDNF (Human, Mouse, Rat) ELISA Kit contains the necessary components required for the quantitative measurement of human, mouse and rat BDNF from serum samples as well as cell culture supernates in a sandwich ELISA format.

This immunoassay contains human BDNF recombinant and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural active BDNF in the samples.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for 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 biotinylated antibody specific for BDNF is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, 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.

## LIMITATIONS OF THE PROCEDURE

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\_ The kit should not be used beyond the expiration date on the kit label.

\_ Do not mix or substitute reagents with those from other lots or sources.

\_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

\_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

\_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

\_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
<b>BDNF Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against BDNF.	<b>752-01-01</b>	<b>1 plate</b>
<b>BDNF Standard</b> – 1500 pg/vial of recombinant human BDNF in a buffered protein base with preservatives; lyophilized.	<b>752-01-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – 105 µL/vial, 100-fold concentrated of Biotinylated antibody against BDNF with preservatives; lyophilized.	<b>752-01-03</b>	<b>1 vial</b>
<b>Positive Control</b> - one vial of recombinant human BDNF, lyophilized	<b>752-01-04</b>	<b>1 vial</b>
<b>Streptavidin-HRP Conjugate</b> -60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	<b>SAHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> - 60mL of buffered protein based solution with preservatives	<b>DB01</b>	<b>1 bottle</b>
<b>Wash Buffer</b> -50 ml of 10-fold concentrated buffered surfactant, with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> -11 ml of TMB substrate solution	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> - 11 ml of 0.5M HCl solution	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1 piece</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1 piece</b>

## STORAGE

**Unopened Kit:** Store at 2 - 8°C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

**Opened / Reconstituted Reagents:** Reconstituted Standard (Stock) and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 12 months.

**Microplate Wells:** Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

**OTHER SUPPLIES REQUIRED**

- Microplate reader capable of measuring absorbance at 450nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

**SAMPLE COLLECTION AND STORAGE**

**Cell Culture Supernates** - Remove particulates by centrifugation and 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 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Due blood platelets have high concentration of BDNF, preparation of plasma samples may affect the platelets to release BDNF. Plasma samples may not be suitable for BDNF assay.

**Note: Use Aprotinin (enzyme inhibitor) (Code No.00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.**

**SAMPLE PREPARATION**

Serum samples may require a 40~80 fold dilution. A suggested 40-fold dilution is 10 µL sample + 390 µL Dilution Buffer. Plasma samples may not suitable for BDNF assay.

**Optimal dilutions should be determined by each laboratory for each application with a sample pretest.**

**Use polypropylene test tubes.**

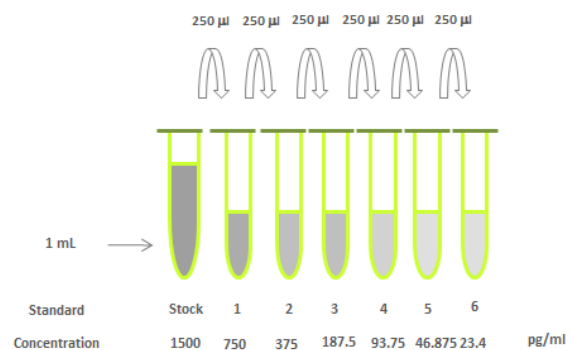
**REAGENT PREPARATION**

**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

**BDNF Standard - Refer to vial label for reconstitution volume.** Reconstitute the **BDNF Standard** with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 1500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tube #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1500 pg/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	1500 pg/ml
# 1	250µl of stock	250µl	750 pg/ml
# 2	250µl of 1	250µl	375 pg/ml
# 3	250µl of 2	250µl	187.5 pg/ml
# 4	250µl of 3	250µl	93.75 pg/ml
# 5	250µl of 4	250µl	46.875 pg/ml
# 6	250µl of 5	250µl	23.4 pg/ml



**Detection Antibody** - Reconstitute the **Detection Antibody concentrated** with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 105 µl of 100-fold concentrated stock solution to prepare working solution.

**Streptavidin-HRP Conjugate** - Pipette 11.94 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 60  $\mu$ L of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

**Positive Control** - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. Positive Control should be prepared and used immediately. Reconstituted Positive Control CAN NOT BE REUSED.

### ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.**

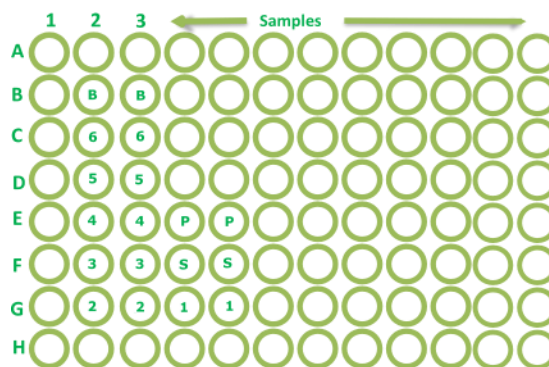
1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100  $\mu$ L of Dilution Buffer to Blank well (B2, B3).
4. Add 100  $\mu$ L of Standard (from C2, C3 to G2, G3 and G4, G5 to F4, F5), sample, or positive control per well (E4, E5). Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300  $\mu$ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100  $\mu$ L of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100  $\mu$ L of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.

10. Add 100  $\mu$ L of Substrate Solution to each well. Incubate for 2-6 minutes at room temperature. **Protect from light.**
11. Add 100  $\mu$ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

### CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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. 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.



### CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human BDNF mature form.

**SENSITIVITY**

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of BDNF was 5-8 pg/ml.

**TYPICAL DATA**

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.085)
2.930	0.032
5.859	0.078
11.7	0.159
23.4	0.254
46.8	0.585
93.75	0.928
187.5	1.433
375	2.033
750	2.830
1500	3.234

**SPECIFICITY**

PROTEINS	CROSS-REACTIVITY
Human BDNF (mature)	100%
Human Pro BDNF (HEK293 derived)	< 1
Human Pro-BDNF (19-128) (E. coli derived)	0
Human Pro-BDNF (19-247) (E. coli derived)	0
Human CNTF	0
Human CTGF	0
Human GRN	0
Human CHGA (19-131)	0
Human NT-3	0

Human Pro BDNF (Glycosylated) derived from HEK293 or CHO cells was indicated less 1% cross-reactive with this elisa kit SK00752-01.

**LINEARITY**

To assess the linearity of the assay, pooled human serum samples were diluted with Dilution Buffer DB01 and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
40 x	481.948	19277.92	100
80 X	239.734	19178.72	99

**SUMMARY OF ASSAY PROCEDURE**

