HUMAN PRO BRAIN-DERIVED NEUROTROPHIC FACTOR (PRO-BDNF) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN PRO-BDNF CONCENTRATIONS IN SERUM.

PURCHASE INFORMATION:

ELISA NAME	HUMAN PRO-BDNF ELISA
Catalog No.	SK00752-06
Lot No.	
Formulation	96 T
Standard range	1.56-100 ng/ml
Sensitivity	0.5 ng/ml
Sample Volume	100 µl
Dilution Factor	*Optimal dilutions should be determined by each laboratory for each application*
Sample Type	Serum
Specificity	Human Pro-BDNF
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	4 °C

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

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INTRODUCTION

Human Pro-BDNF Immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human Pro-BDNF in serum. It contains recombinant human Pro-BDNF and antibodies raised against this protein. It has been shown to accurately quantify recombinant human Pro-BDNF. Results obtained with naturally occurring Pro-BDNF samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the Immunoassay kit can be used to determine relative mass values for natural human Pro-BDNF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for Pro-BDNF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Pro-BDNF present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for Pro-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 Pro-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 the appropriate 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
Pro-BDNF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against Pro-BDNF.	752-06-01	1 plate
Pro-BDNF Standard – 100 ng/vial of recombinant human Pro-BDNF in a buffered protein base with preservatives; lyophilized.	752-06-02	1 vial
Detection Antibody Concentrate– 105 µL / vial, 100-fold concentrated of Biotinylated polyclonal antibody against Pro-BDNF with preservatives; lyophilized.	752-06-03	1 vial
Positive Control- one vial of recombinant human Pro-BDNF, lyophilized	752-06-04	1 vial
Streptavidin-HRP Conjugate -120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Dilution Buffer-10 mL of buffered protein based solution with preservatives	DB02	1 bottle
Antibody & HRP Diluent Solution- 30 mL of buffered protein based solution with preservatives	DB08	1 bottle
Wash Buffer -50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution-11 mL of TMB substrate solution	TMB01	1 bottle
Stop Solution- 11 mL of 0.5M HCl solution	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrated should be stored at -20 or -70 °C. Do not use past kit expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard, Antibody Solution SHOULD BE STORED at -20 °C or – 70°C for up to one months. Streptavidin - HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C.

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

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.

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 not require dilution.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

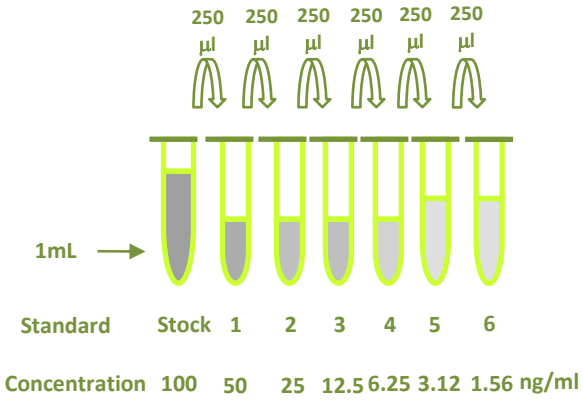
REAGENT PREPARATION

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.

Pro-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 100 ng/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 100 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	100 ng/ml
# 1	250µl of stock	250µl	50 ng/ml
# 2	250µl of 1	250µl	25 ng/ml
# 3	250µl of 2	250µl	12.5 ng/ml
# 4	250µl of 3	250µl	6.25 ng/ml
# 5	250µl of 4	250µl	3.12 ng/ml
# 6	250µl of 5	250µl	1.56 ng/ml



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

Streptavidin-HRP Conjugate - Pipette 11.88 mL of Antibody & HRP Diluent Solution into the 15 ml

centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. Note: 1 x working solution of Streptavidin HRP Conjugate should be used within a few days.

Positive Control- Reconstitute the **Positive Control** with 1 mL of Dilution Buffer. Positive Control should be prepared and used immediately. Reconstituted Positive Control CAN NOT BE REUSED.

ASSAY PROCEDURE

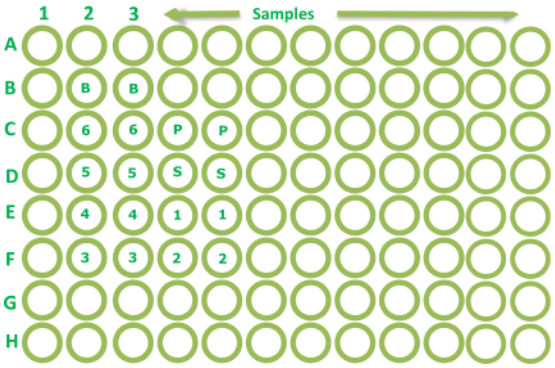
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

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 containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (B2, B3).
4. Add 100 µL of Standard (from C2, C3 to F2, F3 and F4, F5 to D4, D5), sample, or positive control per well (C4, C5). Cover with the 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 µL) 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 µL 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 µL 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 µL of Substrate Solution to each well. Incubate for 18-20 minutes at room temperature. **Protect from light.**
11. Add 100 µL 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, 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 Pro-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 Pro-BDNF.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of Pro-BDNF was 0.5 ng/mL.

TYPICAL DATA

These standard curves* are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD405 (CORRECTED)
Blank	0 (0.146)
1.56	0.026
3.12	0.063
6.25	0.095
12.5	0.200
25	0.310
50	0.487
100	1.109

Lot No.:

Positive Control: 7.31-13.56ng/ml

SPECIFICITY

This assay recognizes both natural and recombinant human Pro-BDNF. The factors listed below were prepared at 10000 ng/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY
Human Pro-BDNF (19-247)	100%
Human BDNF	0
Human CNTF	0
Human CTGF	0
Human GRN	0
Human CHGA (19-131)	0
Human NT-3	0

LINEARITY

To assess the linearity of the assay, pooled human serum samples A were diluted with Dilution Buffer DB08 and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1 x	12.025	12.025	100
2 x	6.848	13.696	114

To assess the linearity of the assay, pooled human serum samples B were diluted with Dilution Buffer DB02 and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1 x	6.238	6.238	100
2 x	2.911	5.822	93.3

The pooled human EDTA plasma samples were tested by this Kit. All samples were under assay limit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 18-20 min on the bench top. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450 nm within 15 min