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

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

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA Name	**PRO-BDNF HUMAN ELISA KIT**
Catalog No.	SK00752-09
Lot No.	
Formulation	96 T
Standard range	0.391 - 25 ng/mL
Sensitivity	0.12 ng/mL
Sample Volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application with a pretest.**
Sample Type	Serum, Plasma
Specificity	Human Pro-BDNF (19-247)
Calibration	Human Pro-BDNF (19-247) Recombinant (HEK293 derived)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 4 months, more information check page 2-3
This kit contains sufficient materials to run approximately 35~40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Pro-BDNF ELISA Kit contains the necessary components required for the quantitative measurement of recombinant human Pro BDNF (19-247) (human cells derived) and/or natural human Pro-BDNF (19-247) from plasma and serum samples in a sandwich ELISA format.

This immunoassay contains human Pro-BDNF (19-247) recombinant and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify bioactive recombinant and natural Pro-BDNF (19-247) in the samples. The mature form BDNF (129-247) or Pro BDNF (19-128) in the samples cannot be detected by this ELISA kit.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human bioactive Pro-BDNF (19-247). The capture antibody can bind to the human bioactive Pro-BDNF in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human Pro-BDNF (19-247) is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human Pro-BDNF (19-247) bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Pro-BDNF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against Pro-BDNF.	**752-09-01**	**1 plate**
Pro-BDNF Standard – 100 ng/vial of recombinant human Pro-BDNF in a buffered protein base with preservative; lyophilized.	**752-09-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of biotinylated monoclonal antibody against Pro-BDNF with preservative; lyophilized.	**752-09-03**	**1 vial**
Positive Control - one vial of recombinant human Pro-BDNF; lyophilized.	**752-09-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer - 45 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08C**	**1 bottle**
Wash Buffer 20X - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB03**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl solution.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C up to 4 months. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C.
Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 ~ 8°C.
Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Plasma and or Serum samples may not require dilution.

Optimal dilutions should be determined by each laboratory for each application with a pretest.
Use polypropylene test tubes.

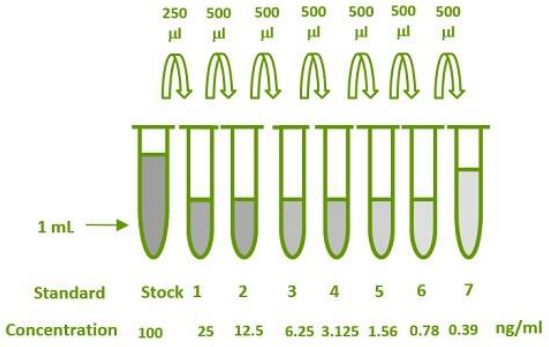
REAGENT PREPARATION

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 **25 mL** of Wash Buffer Concentrate into deionized or distilled water (**475 mL**) to prepare 500 mL of 1x Wash Buffer.

Pro-BDNF Standard - Reconstitute the Pro-BDNF standard with 1.0 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. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **25ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0ml	100 ng/ml
# 1	250 µl of stock	750 µl	25 ng/ml
# 2	500 µl of 1	500 µl	12.5 ng/ml
# 3	500 µl of 2	500 µl	6.25 ng/ml
# 4	500 µl of 3	500 µl	3.125 ng/ml
# 5	500 µl of 4	500 µl	1.56 ng/ml
# 6	500 µl of 5	500 µl	0.78 ng/ml
# 7	500 µl of 6	500 µl	0.39 ng/ml



Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Dilution Buffer (DB01)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Dilution Buffer (DB01)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 10.89 mL of **HRP Diluent Solution (DB08C)** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light).** The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within a few hours.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions, positive control, or samples per well. Cover with the plate sealer. Incubate for **2 hours** on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x 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.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with the plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 15-20 minutes on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 5 min.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-Parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVE

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.085)
0.39	0.031
0.78	0.064
1.56	0.129
3.125	0.269
6.25	0.553
12.5	1.119
25	2.129

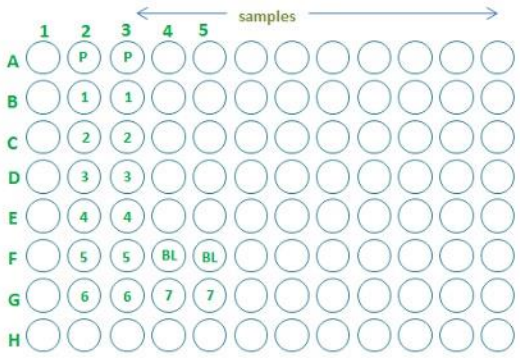
- Lot No.:
- Positive Control: 0.5 – 4 ng/mL

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Pro-BDNF, (HEK293 derived)	100%
Human BDNF, (HEK293 derived)	0
Human BDNF (CHO derived)	0
Human CNTF	0
Human CTGF	0
Human GRN	0
Human CHGA (19-131)	0
Human NT-3	0

The data indicated that human Pro- BDNF (19-247) (*E. Coli* derived) and Human Pro- BDNF (19-128) (*E. Coli* derived) do not have any cross-reactivity with this human pro BDNF ELISA kit.

Well Position



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or 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 15-20 min on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 5 min.