HIGH SENSITIVITY ACTIVE 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	HIGH SENSITIVITY ACTIVE PRO-BDNF HUMAN ELISA KIT
Catalog No.	SK00752-09-HS
Formulation	96 T
Standard Range	62.5 ~ 4000 pg/mL
Sensitivity	10 pg/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). Non Cross-reactivity with human mature BDNF or Pro BDNF (19-128)
Calibration	Human Pro-BDNF 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 High Sensitivity Pro-BDNF Human 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 or 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 human BDNF or human 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 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-HS-01**	**1 plate**
Pro-BDNF Standard – a lot specific per vial of recombinant human Pro-BDNF in a buffered protein base with preservative; lyophilized.	**752-09-HS-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of biotinylated antibody against Pro-BDNF with preservative; lyophilized.	**752-09-HS-03**	**1 vial**
Positive Control - one vial of recombinant human Pro-BDNF; lyophilized.	**752-09-HS-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.	**DB08B**	**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.	**TMB01**	**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 samples may need 2~4 fold dilution for best sample recovery.
A suggested 2-fold dilution is 50 µl per well of sample + 50 µl per well of Dilution Buffer. A suggested 4-fold dilution is 25 µl per well of sample solution + 75 µl per well of Dilution Buffer.
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 20X** 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 stock solution of a lot specific xxx 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 **4000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Store the stock solution at -70 °C for a few days.

TUBE	Standard	Dilution Buffer	Concentration
stock	powder	1.0ml	A lot specific
# 1	xx µl of stock	xxx µl	4000 pg/ml
# 2	250µl of 1	250µl	1000 pg/ml
# 3	250µl of 2	250µl	500 pg/ml
# 4	250µl of 3	250µl	250 pg/ml
# 5	250µl of 4	250µl	125 pg/ml
# 6	250µl of 5	250µl	62.5 pg/ml

Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer. Discard the positive control after use.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Dilution Buffer** to produce a 10-fold concentrated stock solution. For 96 wells test, freshly Pipette 9.45 mL of **Dilution Buffer** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare

working solution. For the partial strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution at -20 °C for a few days.

Streptavidin-HRP Conjugate - For 96 wells test, freshly Pipette 10.89 mL of **HRP Diluent Solution (DB08B)** 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 10-20 min. For the partial strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution at 2 ~ 8 °C for 10 months.

The Pro BDNF Microplate require pre-wash 3 times by filling each well with 1x Wash Buffer (275 µL) prior to perform this ELISA Assay

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 (250 rpm) 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 auto-washer. 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 10-15 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 3 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.

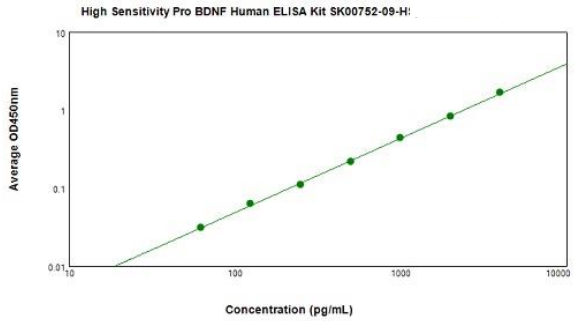
TYPICAL STANDARD CURVE

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

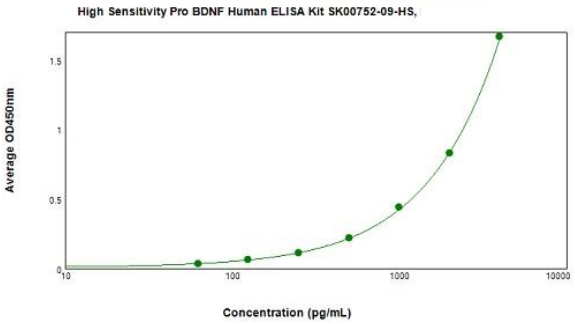
Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.085)
62.5	0.031
125	0.062
250	0.111
500	0.219
1000	0.437
2000	0.829
4000	1.689

- Lot No.:
- Positive Control: refer to a lot specific

Standard curve by log-log fit



Standard curve by 4-parameter fit



SPECIFICITY

Proteins	Cross-reactivity (%)
Human Pro-BDNF (19-247) (HEK293)	100
Mouse Pro-BDNF (19-249) (HEK293)	100
Human Pro BDNF (19-128)	0
BDNF (H, M, R) (CHO)	0
Human CNTF	0
Human NGF	0
Human Pro NGF	0
Human GDNF	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. Mouse EDTA Plasma or serum samples CANNOT be detected by this ELISA Kit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Pre-wash the Pro-BDNF Microplate 3 times. Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate for **2 hours** on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate for 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 10-15 min on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 3 min.

Well Position