ULTRA-SENSITIVITY HUMAN BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) ELISA KIT

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

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. BDNF ARE DETECTABLE IN SALIVA SAMPLES. TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS ASSAY.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	ULTRA-SENSITIVITY HUMAN BDNF ELISA KIT
Catalog No.	SK00752-16A
Lot No.	20115301
Formulation	96 T
Standard range	78 – 5000 fg/mL
Sensitivity	20 fg/mL
Sample Volume	100 µL diluted samples per well
Sample Type	Serum, Plasma, CSF
Dilution Factor	***Optimal dilutions should be determined by each laboratory for each application***
Specificity	Human
Calibration	Human BDNF Tag Free (Dimer, derived from E. Coli)
Intra-assay Precision	4 - 9%
Inter-assay Precision	6 - 12%
Storage	2 - 8° C for 8 months, see page 2 for more information
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 Ultra-Sensitivity Human BDNF Chemiluminescence ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Human BDNF from serum and plasma samples in a sandwich ELISA format. This BDNF ELISA Kit had been formulated to determine the human serum or EDTA plasma or its diluted samples in 100 µL per well.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with an antibody specific for BDNF. The capture antibody can bind to the BDNF in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against BDNF 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 1:1 mixed chemiluminescence substrate solution is added to the wells. Read the luminescence signals by a microplate-reader capable of reading luminescence signal on top. 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.
_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
BDNF Microplate - 96 well white microplate (12 strips of 8 wells) coated with an antibody against BDNF.	**752-16A-01**	**1 plate**
BDNF Standard – 16000 pg/vial of human BDNF in a buffered protein base with preservative; lyophilized.	**752-16A-02**	**vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrated of biotinylated antibody against BDNF with preservative; lyophilized.	**752-16A-03**	**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 **Blue Color** buffered solution with preservative.	**DB93**	**2 bottles**
Antibody Diluent Solution – 12 mL of **Yellow Color** buffered protein based solution with preservative.	**DB0193**	**1 bottle**
HRP Diluent Solution – 12 mL of **Green Color** 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**
Chemiluminescence Substrate Solution A - 6 mL.	**CLSA01**	**1 bottle**
Chemiluminescence Substrate Solution B - 6 mL.	**CLSB01**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 8 months. For longer storage up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and Chemiluminescence Substrate

Solution should be stored only at 2-8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of read luminescence signal on the Top of plate.
- 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) (Aviscera Bioscience's Order Code: 00700-01-25, 25 TIU for 50 ml sample solution) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

If BDNF levels in Human Serum is at 10 ng/mL ~ 24 ng/mL, human serum samples may need 4000 or 8000 fold pre-dilution.
A suggested 20 fold dilution is 5 µL of human serum + 95 µL Dilution Buffer DB93. A 400- fold dilution is 5 µL of 20-fold diluted human serum sample + 95 µL Dilution Buffer DB93. A final 4000-fold dilution is 10 µL of 400-fold diluted human serum sample + 90 µL Dilution Buffer DB93. A final 8000-fold dilution is 5 µL of 400-fold diluted human serum sample + 95 µL Dilution Buffer DB93.

If BDNF levels in Human EDTA Plasma is at 2 ng/mL ~ 5 ng/mL, human serum samples may need 1000~ 2000 fold pre-dilution.
A suggested 10 fold dilution is 10 µL of human plasma + 90 µL Dilution Buffer DB93. A 100- fold dilution is 10 µL of 10-fold diluted human plasma sample + 90 µL Dilution Buffer DB93. A final 1000-fold dilution is 10 µL of 100-fold diluted human serum sample + 90 µL Dilution Buffer DB93. A final 2000-fold dilution is 5 µL of 100-fold diluted human serum sample + 95 µL Dilution Buffer DB93.

The Human Saliva samples can be detected by this ELISA Kit.
TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS 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 **25 mL of Wash Buffer Concentrate 20X** into deionized or distilled water (**475 mL**) to prepare 500 mL of 1x Wash Buffer.

BDNF Standard - Reconstitute the BDNF standard with 1 mL of **Blue Color Dilution Buffer DB93**. This reconstitution produces a stock solution of 16000 pg/mL. Allow the standard to sit for a minimum of 3 minutes with gentle agitation prior to making dilutions. Pipette 990 µL of Dilution Buffer DB93 into the tube sub-stock. Pipette 700 µL of Dilution Buffer DB93 into the tube #1. Pipette 300 µL of Dilution Buffer DB93 into the tube #2 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **5 pg/mL** standard serves as the high standard. The Dilution Buffer DB93 **Blue Color** serves as the zero standard (0 pg/mL). Only **Blue Color** 100 µL per well of each standard solution for human BDNF assay.

Store the stock solution of standard at -20 ~ - 70 °C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	16000 pg/ml
Sub-stock A	10 µl of stock	990 µl	160 pg/ml
Sub-stock B	100 µl of sub-stock A	700 µl	20 pg/ml
# 1	100 µl of sub-stock B	300 µl	5 pg/ml
# 2	100 µl of 1	300 µl	1.25 pg/ml
# 3	100 µl of 2	300 µl	0.312 pg/ml
# 4	100 µl of 3	300 µl	0.078 pg/ml

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **yellow color Antibody Diluent Solution (DB0193)** to produce a 10-fold concentrated stock solution. For 96 wells test, freshly pipette 9.9 mL of **Antibody Diluent Solution (DB0193)** into a 15 ml centrifuge tube and transfer 1.1 mL of 10-fold concentrated stock solution to prepare working solution. Only 100 µL per well of each detection antibody solution (**yellow color**). The 1 x working solution should be used in 10 ~ 20 min. *Store the stock solution of 10-fold concentrated detection antibody at -20 °C for a few days. If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution.*

Streptavidin-HRP Conjugate – For 96 wells test freshly pipette 11.88 mL of **HRP Diluent solution (DB08C) Green Color** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution (**protect from light**). 100 µL per well of working solution (**Green Color**).

The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within 10 min. If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 100-fold concentrated Streptavidin HRP at 2 -8°C for 12 months.

1:1 mixed **Color Less 5.5 mL of each Chemiluminescence Substrate Solution A** and **B** for 96 wells test prior to use. Add 1:1 mixed **100 µL of each Substrate Solution A** and **B** per well. For partial strips test, Total 900 µL per 8-well strip of 1:1 mixed solution prior to use.

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. **Pre-wash the BDNF Microplate 752-16-01 with 1 x Wash Buffer 275µL per well for 3 times prior to assay.**
2. Add **100 µL** per well of Dilution Buffer to Blank wells.
3. Add **100 µL of standard** dilutions in reverse order of serial dilution, **100 µL of samples or diluted samples** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Discard the solution of each well by hold the edge of plate and quickly invert the plate top down. Discard and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (275µL) using a 8 channel pipette. Complete removal of liquid at each step is essential to good performance.
5. **Add 100 µL of Detection Antibody working solution to each well**. Cover with plate sealer. Incubate for 90 minutes on microplate shaker at room temperature.
6. Repeat the Discard/wash as in step 4.
7. Add **100 µL** of Streptavidin-HRP Conjugate working solution (Green Color) to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the discard/wash as in step 4.
9. Add Color Less **100 µL** of 1:1 Pre-mixed Substrate Solution A and B to each well. **Protect from light.**
10. Read the luminescence signals by a microplate-reader capable of reading luminescence signal on the top of plate at 2 min and or 12 min . If the luminescence signal is over read limit, ready the plate at 30 min or 45 min.
    Set up Luminescence Read:
    Integration Time = 400 ms.
    Read from Top.
    Read Heights 5.45 mm.
    shake the plate for 3~ 4 sec prior to the first read.

Read Order: Row.

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 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.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human BDNF (Dimer) (E. Coli derived)	100
Human BDNF (CHO derived)	100
Human Pro BDNF (19-247)(HEK293 derived)	<1
Mouse Pro BDNF (19-249) (HEK293 derived)	<1
Human Pro-BDNF (19-128) (E. Coli derived)	0
Human CNTF	0
Human CTGF	0
Human NT-3	0

Goat or Bovine samples may detectable by this ELISA Kit. Please use animal free cell cultures or animal free buffer solution for BDNF assay. Mouse Sample can not be tested by this human BDNF ELISA Kit. Rat serum or plasma samples were tested by this elisa kit.

Human Pro-BDNF (glycosylated) (19-247) R127A, R128A derived from HEK293 were diluted by Dilution Buffer DB93 at 50ng/mL, 25 ng/mL, 6.25 ng/mL and 1.56 ng/ml. The Human Pro BDNF (HEK293) indicated to have less than 1% cross-reactivity with this ELISA kit SK00752-16A.

TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed. Read RLU at 12 min.

STANDARD (FG/ML)	RLU (CORRECTED)
Blank	0 (5.5e4)
78	2139
312.5	9834
1250	41541
5000	169763

- Lot No.: 20115301

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Pre-wash the BDNF Microplate for 3 times prior to assay. Add **100 µl** of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
⬇
Discard and wash 4 times.
⬇
Add **100 µl** Detection Antibody working solution to each well. Incubate 90 minutes on the plate shaker at RT.
⬇
Discard and wash 4 times.
⬇
Add **100 µl** Streptavidin-HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. **Protect from light**.
⬇
Discard and wash 4 times.
⬇
Add 100 µl 1:1 mixed Chemiluminescence Substrate Solution A and B to each well. Read the luminescence signals on the top of plate at 2 min or 12 min, or 30 min.

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Human Raptin/RCN2 ELISA Kit	SK00168-18
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Human Endotrophin ELISA Kit	SK00009-06
Mouse Endotrophin ELISA Kit	SK00009-03
HS Mouse CSF1R ELISA Kit	SK00144-03
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