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

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**BDNF (HUMAN, RAT) ELISA KIT**
Catalog No.	SK00752-01C
Lot No.	
Formulation	96 T
Standard range	23 - 1500 pg/mL
Sensitivity	4 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma,
Dilution Factor	40~160 (***Optimal dilutions should be determined by each laboratory for each application)***
Specificity	BDNF mature form at 100%, human Pro-BDNF derived from human cells at less than 2%
Calibration	Mature human BDNF (CHO derived)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
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.	

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.

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DESCRIPTION

This BDNF (Human, Rat) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural BDNF from serum and plasma samples in a sandwich ELISA format.

This immunoassay contains recombinant BDNF and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural mature 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 substrate solution is added to the wells and color develops in direct proportion to the amount of BDNF 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.
_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 polystyrene microplate (12 strips of 8 wells) coated with an antibody against BDNF.	**752-01C-01**	**1 plate**
BDNF Standard – 1500 pg/vial of rh BDNF (CHO derived) in a buffered protein base with preservative; lyophilized.	**752-01C-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrated of biotinylated antibody against BDNF with preservative; lyophilized.	**752-01C-03**	**1 vial**
Positive Control- one vial of recombinant BDNF; lyophilized.	**752-01C-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 - 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 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 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) (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

Human Serum samples may require at least a 40 ~ 160 fold. A suggested 40-fold dilution is 10 µl sample + 390 µl Dilution Buffer. A suggested 80-fold dilution is 50 µl per well of 40-fold diluted sample solution + 50 µl per well of Dilution Buffer. A suggested 160-fold dilution is 25 µl per well of 40-fold diluted sample solution + 75 µl per well of Dilution Buffer.

Human Plasma samples may require 8 ~ 32 fold dilution or run a pre-test to check the optimal dilution factors.
Rat serum samples may require 10 ~ 40 fold dilution or run a pre-test to check the optimal dilution factors.

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

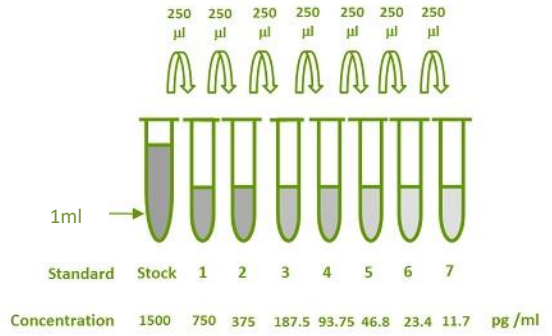
Use polypropylene test tubes.

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

BDNF Standard - Reconstitute the BDNF standard with 1.0 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 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 Dilution Buffer serves as the zero standard (0 pg/mL). Optional set the 11.72 pg/mL as the lowest standard concentration. Store the stock solution of standard at -20 ~ - 70 °C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.5 ml	1500 pg/ml
# 1	250 µl of stock	250 µl	750 pg/ml
# 2	250µl of 1	250 µl	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	46.88 pg/ml
# 6	250µl of 5	250µl	23.44 pg/ml
#7 (optional)	250µl of 6	250µl	11.72 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. Discard the positive control after use. It is for one time use only.

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. *If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 10-fold concentrated detection antibody at -20 °C for a few days.*

Streptavidin-HRP Conjugate – For 96 wells test freshly pipette 11.88 mL of **HRP Diluent solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 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 20-30 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.

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 in reverse order of serial dilution, samples, or positive control per well. Cover with 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 plate sealer. Incubate for 90 minutes 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 45 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 2 minutes.

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 or 4-parameter 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 (mature) (CHO derived)	100
Human BDNF (mature) (HEK293 derived)	100
Human BDNF (Dimer) (E. Coli derived)	100
Human Pro BDNF (HEK293 derived)	< 2
Human Pro-BDNF (19-128) (E. Coli derived)	0
Human Pro-BDNF (19-247) (E. Coli derived)	0
Human CNTF	0
Human NT-3	0

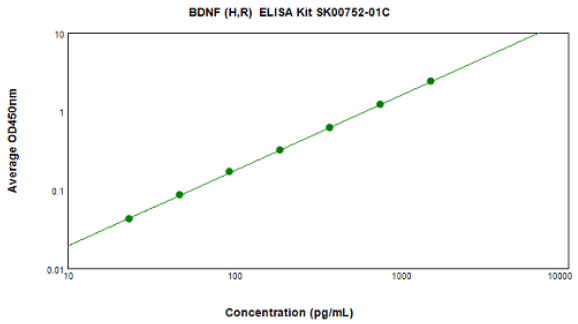
The data indicated BDNF in Mouse Serum or EDTA plasma samples were not detectable by this elisa kit. Human Pro-BDNF (glycosylated) derived from HEK293 were sparked to mouse serum samples at 100ng/mL and 50 ng/mL. The cross-reactivity was assayed by SK00752-01C at less than 2% cross-reactivity with this ELISA kit SK00752-01C.

TYPICAL STANDARD CURVE

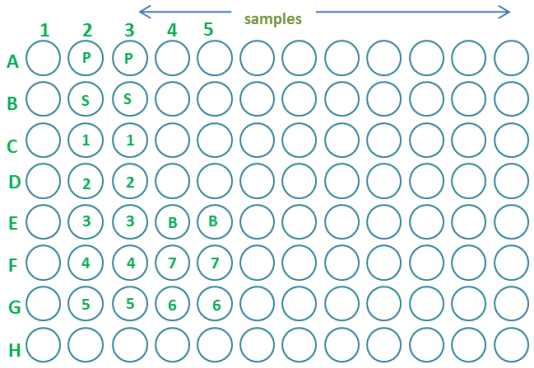
This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.064)
11.72 (optional)	0.030
23.44	0.056
46.88	0.099
93.75	0.189
187.5	0.367
375	0.712
750	1.419
1500	2.430

- Standard curve by log-log fit



Well Position



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the 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 2 min.

PERFORMANCE CHARACTERISTICS

Precision:

Intra-assay Precision (Precision within an assay)- Three samples of known concentration were tested fourteen times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)- Three samples of known concentration were tested three times on one plate to assess intra-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
N	14	14	14	3	3	3
Mean (pg/mL)	96	192	390	97	198	398
SD	4.322	10.091	18.848	14.271	14.179	20.142
CV%	3.9	5.2	4.5	3.9	5.9	7.493

Recovery: The recovery of human BDNF spiked to different levels throughout the range of the assay in related matrices was evaluated by BDNF (Human, Rat) ELISA Kit SK00752-01C.

Sample	Average of Recovery	Range of Recovery
Human Serum	105%	102% ~ 108%

Linearity:

Human Serum	Average of Recovery	Range of Recovery
1:40 Diluted	106%	104% ~ 108%
1:80 Diluted	105%	102% ~ 108%
1:160 Diluted	105%	102% ~ 108%

Sensitivity: 4 pg/mL which was determined by adding two standard deviations to the 20 zero standard replicates and calculating the corresponding concentration.

Research Sample Test

The research pooled samples were diluted by Dilution Buffer DB01 and assayed by BDNF (Human, Rat) ELISA Kit SK00752-01C.

Sample	Dilution Factor	Assayed (pg/ml)	Final (ng/ml)	Recovery (%)
Human Serum F	40	432.391	17.295	100
Human Serum F	80	219.449	17.555	102
Human Serum F	160	111.082	17.773	103

The research pooled human serum or EDTA plasma samples were diluted by Dilution Buffer DB01. Its linearity and recovery was assayed by BDNF ELISA Kit SK00752-01C.

Sample	Dilution Factor	Assayed (pg/ml)	Final (ng/mL)	Recovery (%)
Serum	40	567.305	22.692	100
Serum	80	297.726	23.818	105
Serum	160	165.008	26.401	116
EDTA Plasma	8	725.206	5.802	100
EDTA Plasma	16	332.118	5.314	92
EDTA Plasma	32	167.114	5.348	92

The research pooled rat serum samples were diluted by Dilution Buffer DB01. Its linearity and recovery was assayed by BDNF ELISA Kit SK00752-01C.

Sample	Dilution Factor	Assayed (pg/ml)	Final (ng/mL)	Recovery (%)
Rat Serum	10	243.530	2.435	100
Rat Serum	20	125.127	2.502	103
Rat Serum	40	57.328	2.293	94