Mouse BDNF ELISA Kit

Catalog Number：RK00433

This ELISA kit used for quantitative determination of mouse Brain-Derived Neurotrophic Factor (BDNF) concentration in cell culture supernate, serum and plasma. For research use only, and it's highly recommended to read throughly of this manual before using the product.

Manufactured by

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Introduction

Mouse mature BDNF is a 13 kDa, 119 amino acid (aa) residue non-glycosylated polypeptide whose primary structure is conserved among all mammalian species examined (1-4). Initially synthesized as a 247 aa residue prepropeptide, the BDNF molecule is divided into an 18 aa residue signal sequence, a 110 aa residue prosequence, and a 119 aa residue mature segment (2). Similar to other neurotrophic factors, there is a possibility that the N-terminus is alternatively spliced, giving rise to a longer pre-pro segment (but identical mature segment) with different functional properties (2). As a mature molecule, BDNF is 52% identical to NGF at the amino acid level, exists as a noncovalently-linked homodimer in solution, and contains six cysteine residues that are believed to form three intrachain disulfide linkages (1-3). Cells known to express BDNF include fibroblasts (5), astrocytes (6), neurons of varying phenotype and location (6-8), megakaryocytes/platelets (9), Schwann cells (near injury) (10) and, possibly, smooth muscle cells (11). BDNF in plasma is detected in the pg/mL range, while BDNF in serum is measured in the ng/mL range, the difference apparently attributable to platelet degranulation and BDNF release during clotting (3, 9, 12). The conservation of BDNF structure potentially allows a mouse BDNF ELISA to be widely applied across species.

There are at least two receptors for BDNF, the first being the low affinity 75 kDa Nerve Growth Factor Receptor (LNGFR), and the second being the high affinity 145 kDa TrkB (tropomyosin receptor kinase-B) (13). The LNGFR is a 399 aa residue Type-I (extracellular N-terminus) transmembrane glycoprotein that is currently considered to be a member of the TNF receptor superfamily (14, 15). Although all neurotrophins bind LNGFR with approximately the same affinity (Kd~1 nM), the significance of such binding is uncertain (13, 16, 17). What seems

clear is that the LNGFR alone can engage certain signal transduction pathways (18). The biological significance of the activation of these pathways is not well understood. For BDNF specifically, LNGFR may serve as a retrograde transport molecule in neurons, promote Schwann cell migration near injury, and/or modulate TrkB activity in those cells that co-express both LNGFR and TrkB (16, 18). The second receptor for BDNF is the TrkB high affinity receptor (Kd~10 pM), a receptor that also binds NT-3 and NT-4/5 (13, 19). Mouse TrkB is a 792 aa residue type I (extracellular N-terminus) transmembrane glycoprotein that exhibits a number of distinct extracellular domains. These include two N-terminal cysteine-rich regions that flank an intervening leucine-rich domain and two membrane proximal C2 Ig-like domains (20). Comparing mouse and rat proteins, these regions exhibit over 90% identity in aa sequence (20). Alternative splice events have been identified for the TrkB gene in the mouse (21), the rat (22), and the mouse (20). In each case, nonsignalling, cytoplasmically-truncated variants are produced, leading to speculation that alternative splicing may be one method by which cells down-regulate neurotrophin activity (20, 22). While full TrkB activity is believed to require TrkB homodimerization (22), evidence suggests that full length TrkB and TrkC receptors may also form functional heterodimers in select cells where both receptors are co-expressed. These include cerebellar granule neurons and neurons of the hippocampal dentate nucleus (23, 24). Among the cells known to express TrkB are motoneurons of the spinal cord (10), pyramidal cells of the hippocampus (25), almost all neurons in the developing brain (25), and thymocytes (26), leading to speculation that BDNF plays a role in lymphopoiesis.

The number of functions attributed to BDNF is quite large. During development, BDNF has been implicated in neuronal differentiation, maturation, survival and synapse formation (27). In the adult, one of its most promising roles centers on

neuroprotection, possibly protecting forebrain neurons from ischemic attack (28) and motor neurons from axotomy-induced death (29).

Principle Of The Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for BDNF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BDNF present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for BDNF is added to the wells and binds to the combination of capture antibody-BDNF in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added. A colored product TMB is formed in proportion to the amount of BDNF present in the sample. The reaction is terminated by addition of acid and absorbance is measured. A standard curve is prepared from seven BDNF standard dilutions and BDNF sample concentration determined.

Materials Provided

Part	Size (96T)	Cat NO.	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Antibody Coated Plate	**8×12**	**RM01761**	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 °C.Reseal along entire edge of zip-seal.
Standard Lyophilized	**2**	**RM01758**	Aliquot and store at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Concentrated Biotin Conjugate Antibody (100×)	**1 ×120ul**	**RM01759**	May be stored for up to 6 month at-20 °C.*
Streptavidin-HRP Concentrated (100×)	**1 ×120ul**	**RM01760**	
Standard/Sample Diluent (R1)	**1 ×20mL**	**RM00023**	May be stored for up to 6 month at 2-8 °C.*
Biotin-Conjugate Antibody Diluent (R2)	**1 ×12mL**	**RM00024**	
Streptavidin-HRP Diluent(R3)	**1 ×12mL**	**RM00025**	
Wash Buffer(20x)	**1 × 30mL**	**RM00026**	
TMB Substrate	**1 ×12 mL**	**RM00027**	
Stop Solution	**1 ×6 mL**	**RM00028**	
Plate Sealers	**4 strips**		
Specification	**1**		

Sample Collection And Storage

1. **Cell Culture Supernates:**

   Centrifuge 1000x g for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.If cell culture supernate samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the Standard/Sample Diluent(R1).

2. **Serum:**

   Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.

3. **Plasma**

   Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze / thaw cycles.

4. **Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.**

5. **Dilution:**

   Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

Precautions

1. **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Apart from the standard of kits, other components should not be refrigerated.
7. Please perform simple centrifugation to collect the liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
10. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and the sequence of the regents should be added consistently.
12. Reuse of dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
16. To avoid cross contamination, please use disposable pipette tips.

17. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
18. The 48T kit is also suitable for the specification.

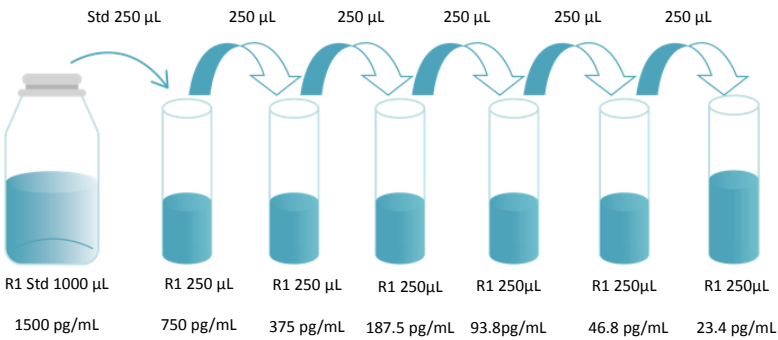
Experiment Materials

1. Microplate reader(measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips：0.5-10, 2-20, 20-200, 200-1000 μL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water, graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

Reagent Preparation

1. **Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.**

2. **Standard:** Add Standard/Sample Diluent(R1) 1.0mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (1500pg/mL), Prepare EP tubes containing Standard/Sample Diluent(R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 1500, 750, 375, 187.5, 93.75, 46.875, 23.4375, 0 pg/mL). Redissolved standard solution (1500 pg/mL), aliquot and store at -20°C— -70°C.



3. **Concentrated Biotin Conjugate Antibody (100x)** : Dilute 1:100 with the Biotin-Conjugate Antibody Diluent (R2) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated Biotin-Conjugate antibody (100x)	Biotin-Conjugate Antibody Diluent (R2)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

4. **Streptavidin-HRP Concentrated (100x):** Dilute 1:100 with the Streptavidin-HRP Diluent(R3) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated Streptavidin-HRP (100x)	Streptavidin-HRP Diluent(R3)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

5. **Wash buffer**: Dilute 1:20 with double distilled or deionized water before use.

Wash Method

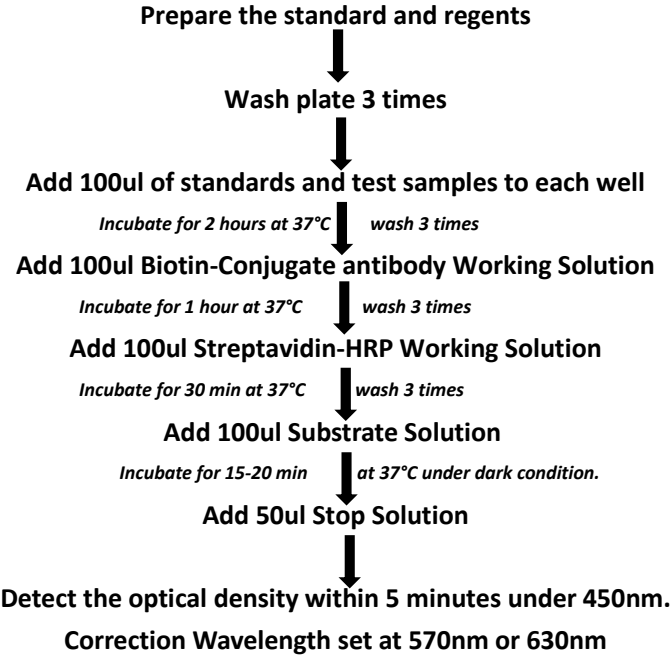
Aspirate each well and wash, repeating the process two times for a total of three washes.Wash by filling each well with **Wash Buffer**(300ul) using a squirt bottle,manifold dispenser,or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash,remove any remaining **Wash Buffer** by aspirating or decanting.Invert the plate and blot it against clean paper towels.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add wash buffer 300 µL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
3. Add 100 µL Standard/sample Diluent (R1) in blank well.
4. Add 100 µL different concentration of standard and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at 37°C.
5. Repeat the aspiration/wash as in step 2.
6. Prepare the Concentrated Biotin Conjugate Antibody (100X) Working Solution 15 minutes early before use.
7. Add Biotin-Conjugate antibody Working Solution in each wells (100µL/well), cover with new adhesive strip provided. Incubate for 1 hour at 37°C.
8. Prepare the Streptavidin-HRP Concentrated (100X) Working Solution 15minutes early before use.
9. Repeat the aspiration/wash as in step 2.
10. Add Streptavidin-HRP Working Solution in each wells (100 µL/well), cover with new adhesive strip provided. Incubate for 30 minutes at 37°C.
11. Warm-up the Microplate reader.
12. Repeat the aspiration/wash as in step 2.
13. Add TMB Substrate (100µL/well). Incubate for 15-20 minutes at 37°C .Protect from light.
14. Add Stop Solution (50µL/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at

450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

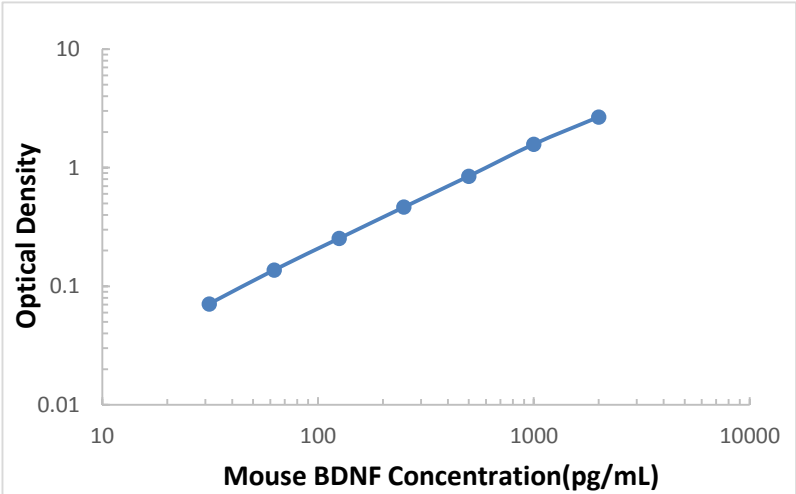
Assay Procedure Summary



Calculation Of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. 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 a log/log graph. The data may be linearized by plotting the log of the BDNF concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted,the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of BDNF assayed.

Sensitivity

The minimum detectable dose (MDD) of BDNF is typically less than 6.8 pg/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes both recombinant and natural Mouse BDNF. The factors listed below were prepared at 50ng/ml and assayed for cross-reactivity. No significant cross-reactivity was observed with the following:

Recombinant mouse:	Recombinant human:
Pro-BDNF(aa19-128)	CNTF
Beta-NGF	Pro-BDNF(aa19-128)
TrKC	Beta-NGF
TrKB	NGF R
NGF R	NT-3
	NT-4

Precision

Intra-plate Precision

Three samples of known concentration were tested 20 times on one plate to evaluate the Intra-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	86	436	865
Standard Deviation (SD)	3.09	12.2	26.8
Variable Coefficient CV (%)	3.6	2.8	3.1

Inter-plate Precision

Three samples of known concentration were tested 20 times separate assays to evaluate the Inter-plate precision.Assay were using two lots of components.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	90	456	900
Standard Deviation (SD)	6.12	34.6	55.8
Variable Coefficient CV (%)	6.8	7.6	6.2

Recovery

Spike 3 different concentration of mouse BDNF into healthy mouse serum and plasma, calculate the recovery.

Sample Form	Average Recover (%)	Range (%)
Serum	102	90-114
Plasma	100	92-108

Linearity

Spike high concentration of mouse BDNF into 4 healthy mouse serum, dilute in the range of standard curve kinetics and evaluate the linearity.

Dilution	Average Value (%)	Range (%)
1:2	98	90-106
1:4	95	86-104
1:8	95	84-105
1:16	100	91-109

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