PRODUCT INFORMATION & MANUAL

Human/Mouse BDNF Valukine™ ELISA

VAL136

For the quantitative determination of natural and recombinant human/mouse BDNF concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version202305.3

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I. BACKGROUND

Brain-derived neurotrophic factor (BDNF) is a member of the NGF family of neurotrophic factors (also named neurotrophins) that are required for the differentiation and survival of specific neuronal subpopulations in both the central as well as the peripheral nervous system. The neurotrophin family is comprised of at least four proteins including NGF, BDNF, NT-3, and NT-4/5.

Human 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. 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). High levels of expression of BDNF have been detected in the hippocampus, cerebellum, fetal eye and placenta. In addition, low levels of BDNF expression are also found in the pituitary gland, spinal cord, heart, lung and skeletal muscle.

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) (12). The number of functions attributed to BDNF is quite large. During development, BDNF has been implicated in neuronal differentiation, maturation, survival and synapse formation (13). In the adult, one of its most promising roles centers on neuroprotection, possibly protecting forebrain neurons from ischemic attack (14) and motor neurons from axotomy-induced death (15).

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, 16). The conservation of BDNF structure potentially allows a human BDNF ELISA to be widely applied across species.

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human/mouse BDNF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human/mouse BDNF present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human/mouse BDNF are pipetted into the wells. After washing away any unbound substances, streptavidin-HRP are pipetted into the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of BDNF bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate and serum.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	101.0	278.8	898.0	102.0	279.5	905.5
Standard Deviation	2.2	7.4	26.6	3.2	7.7	41.9
CV%	2.1	2.7	3.0	3.2	2.8	4.6

B. RECOVERY

The recovery of human/mouse BDNF spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range**
Cell culture media (n=4)	94	86 - 104%
Human Serum (n=5)	108	101 - 112%
Mouse Serum (n=5)	91	86 - 98%

C. SENSITIVITY

The minimum detectable dose (MDD) of human/mouse BDNF is typically less than 1.552 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.

D. CALIBRATION

This immunoassay is calibrated against highly purified *E.coli*-expressed recombinant human/mouse BDNF produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human/mouse BDNF in various matrices and diluted with Diluent 1× to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=4)	Human Serum (n=5)	Mouse Serum (n=5)
1:2	Average % of Expected	92	96	93
	Range (%)	82 - 103	93 - 101	90 - 103
1:4	Average % of Expected	105	98	100
	Range (%)	84 - 114	93 - 101	90 - 111
1:8	Average % of Expected	101	110	107
	Range (%)	87 - 113	105 - 113	96 - 116
1:16	Average % of Expected	101	110	106
	Range (%)	87 - 110	106 - 117	97 - 119

F. SAMPLE VALUES

Human Serum - Five human serum samples were evaluated for the presence of human/mouse BDNF in this assay. All samples measured ranged from 9125 to 20667.5 pg/mL with an average of 15589.3 pg/mL.

Mouse Serum - Five mouse serum samples were evaluated for the presence of human/mouse BDNF in this assay. All samples measured less than the lowest human/mouse BDNF standard, 23.4 pg/mL.

G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

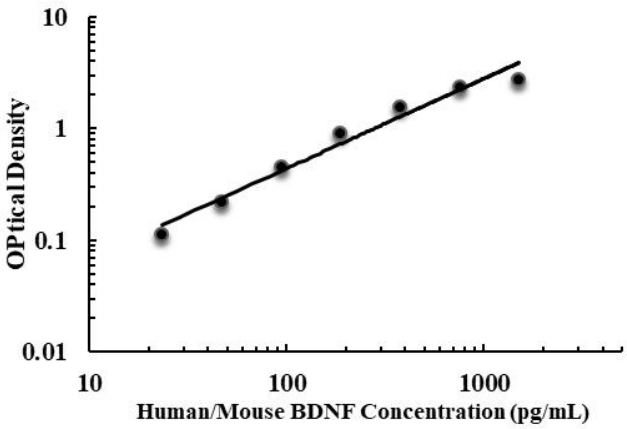
Recombinant human	
B-NGF	NT-3
GDNF	NT-4

Recombinant human TrkB/Fc Chimera does not cross-react in this assay, but does interfere at concentrations > 100 pg/mL.

IV. EXPERIMENT

EXAMPLE STANDARD

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



pg/mL	OD	Average	Corrected
0	0.059 0.057	0.058	-
23.4	0.170 0.172	0.171	0.113
46.9	0.284 0.271	0.278	0.220
93.8	0.527 0.498	0.513	0.455
187.5	1.006 0.949	0.978	0.920
375	1.648 1.616	1.632	1.574
750	2.442 2.414	2.428	2.370
1500	2.827 2.836	2.832	2.774

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human/Mouse BDNF Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against human/mouse BDNF.	1 plate
Human/Mouse BDNF Standard	Recombinant human/mouse BDNF in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human/Mouse BDNF Detection Antibody	Biotinylated BDNF antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (1×)	Buffered diluent used to dilute standard and samples.	2 vials
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent (10×)	A 10× concentrated buffered protein base used to dilute Detection Antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8 °C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP A</td><td rowspan="3">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent (1×)</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature 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.

B. SAMPLE PREPARATION

Cell Culture Supernates samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×).

Human Serum samples require a 100-fold dilution. For example, add 10 µL of human serum into a tube with 90 µL Calibrator Diluent (1×) to prepare a 10-fold diluted sample. Mix through and then pipette 10 µL of prepared 10-fold diluted sample into a tube with 90 µL Calibrator Diluent (1×) to prepare a final 100 fold diluted sample.

Mouse Serum samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

Note: *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 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

Reagent Diluent (1×) - Use deionized or distilled water to prepare Reagent Diluent (1×).

Detection Antibody (1×) - Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100×). Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection Antibody (1×) with Reagent Diluent (1×). Prepare at least 15 minutes prior to use.

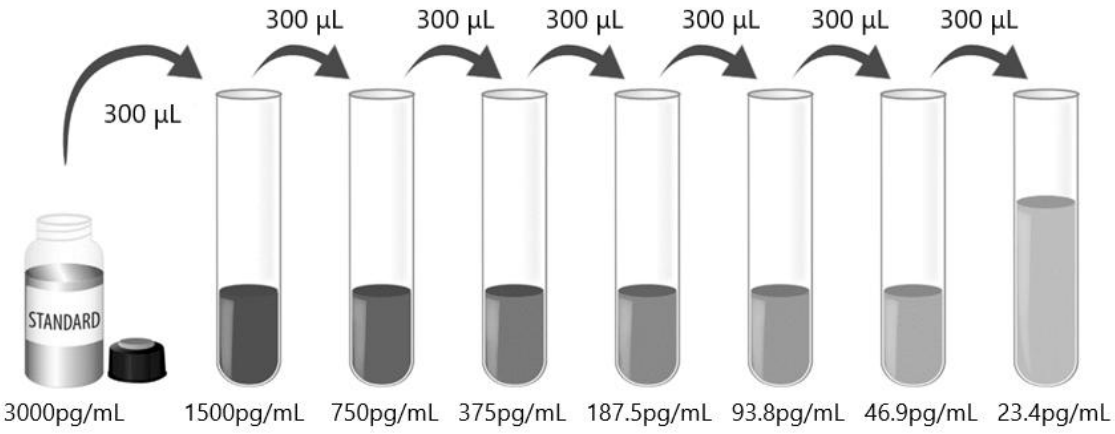
Streptavidin-HRP A (1×) **- Centrifuge briefly before opening.** Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Human/Mouse BDNF Standard - Centrifuge briefly before opening. Refer to the

vial label for the reconstitution volume*. This reconstitution produces a stock solution of 3000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*if you have any question, please seek help from our Technical Support.

Pipette 300 µL of the appropriate Calibrator Diluent (1×) into each tube. 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 Calibrator Diluent (1×) serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

VII. ASSAY PROCEDURE

Note: *Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of standard, or prepared sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Solution (400 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 the Detection Antibody (1×) diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 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.

12. CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph. The data may be linearized by plotting the log of the BDNF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

VIII. REFERENCES

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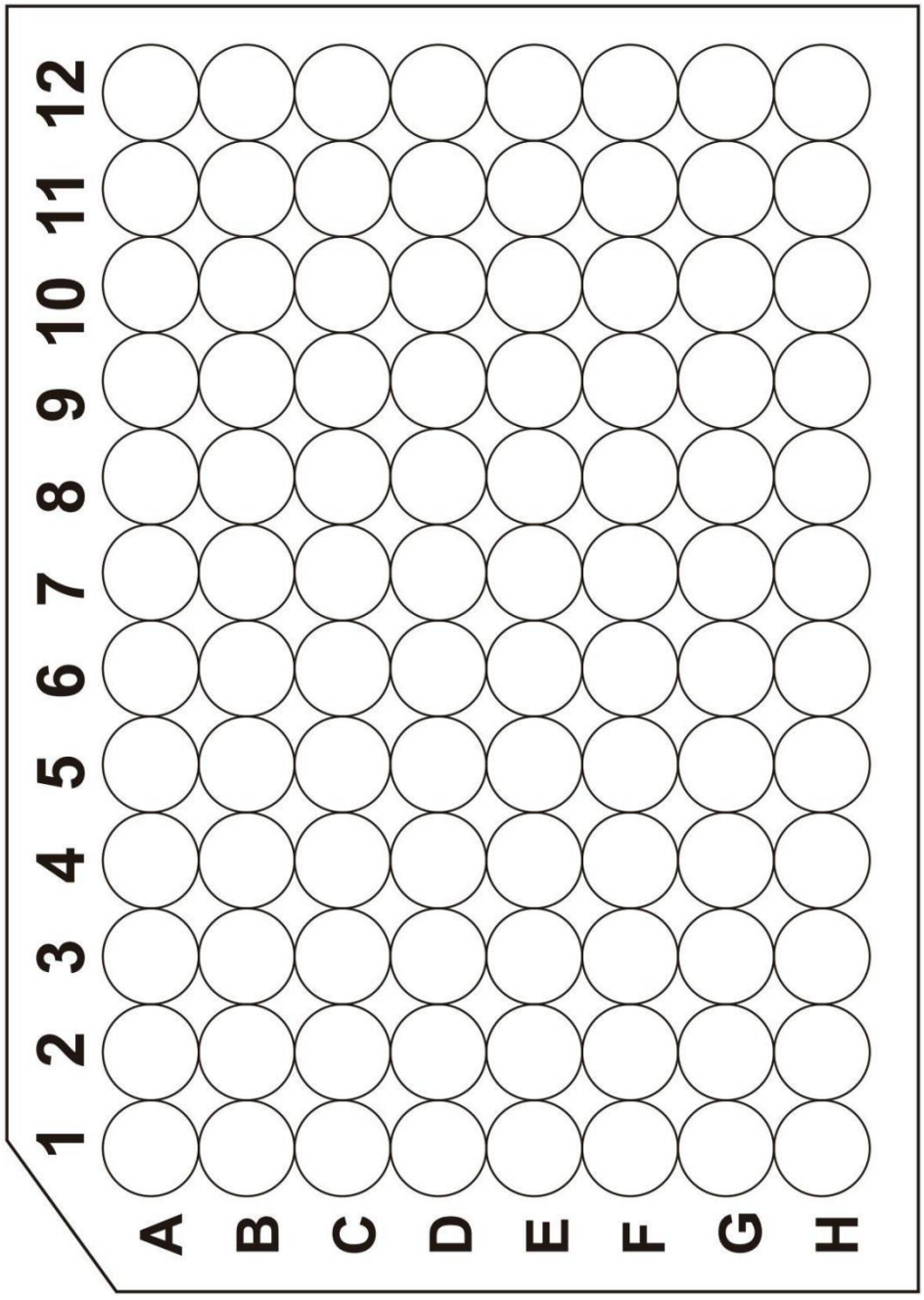
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人/小鼠 BDNF Valukine™ ELISA 试剂盒

目录号：VAL136

适用于定量检测天然和重组人/小鼠 BDNF 的浓度

科研专用，不可用于临床诊断

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目录

I. 背景

脑源性神经营养因子（BDNF）是神经营养因子NGF家族成员，是中枢神经系统和外周神经系统中特定神经元亚群分化和存活所必需的。神经营养因子家族至少由四种蛋白质组成，包括NGF、BDNF、NT-3和NT-4/5。

人类成熟的BDNF是一种13kda，119个氨基酸（aa）残基组成的非糖基化多肽，其一级结构在所有被检测的的哺乳动物中都是保守的（1-4）。BDNF分子最初合成一个247 aa残基的前肽，分为18 aa残基信号序列、110 aa残基前序列和119 aa残基成熟片段。与其他神经营养因子类似，N-末端可能是交替拼接的，从而产生一个较长的具有不同的功能特性的pre-pro片段（而成熟片段则相同）（2）。作为一个成熟的分子，BDNF在氨基酸水平上与NGF有52%的同源性，在溶液中以非共价连接的同二聚体形式存在，并含有六个半胱氨酸残基，它们被认为可形成三个链内二硫键（1-3）。已知表达BDNF的细胞包括成纤维细胞（5）、星形胶质细胞（6）、不同表型和位置的神经元（6-8）、巨核细胞/血小板（9）、雪旺细胞（近损伤）（10）和可能的平滑肌细胞（11）。BDNF在海马、小脑、胎眼和胎盘中有高水平表达。此外，在垂体、脊髓、心脏、肺和骨骼肌中也发现低水平的BDNF表达。

BDNF至少有两种受体，第一种是低亲和力75kDa神经生长因子受体（LNGFR），第二种是高亲和力145kDa TrkB（原肌球蛋白受体激酶B）（12）。BDNF的功能非常多。在发育过程中，BDNF参与了神经元的分化、成熟、存活和突触的形成（13）。在成人中，它最有希望的作用之一是神经保护，可能保护前脑神经元免受缺血性攻击（14）和运动神经元免受轴突切除引起的死亡（15）。

血浆中的BDNF的检测范围为pg/mL，而血清中BDNF检测范围为ng/mL，这种差异明显归因于凝血过程中血小板脱颗粒和BDNF释放（3, 9, 16）。BDNF结构的保存有可能使人BDNF-ELISA在物种间广泛应用。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人/小鼠BDNF抗体包被于微孔板上，样品和标准品中的人/小鼠BDNF会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人/小鼠BDNF检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶(streptavidin-HRP)孵育。洗涤去除未结合的物质后，加入TMB底物溶液，溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本和血清样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释剂（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的储存等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板中分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	101.0	278.8	898.0	102.0	279.5	905.5
标准差	2.2	7.4	26.6	3.2	7.7	41.9
CV%	2.1	2.7	3.0	3.2	2.8	4.6

B. 回收率

在不同类型样本中掺入检测范围内不同水平的人或者小鼠 BDNF，测定其回收率。

样本类型	平均回收率	范围
细胞培养上清 (n=4)	94	86 - 104%
人血清 (n=5)	108	101 - 112%
小鼠血清 (n=5)	91	86 - 98%

C. 灵敏度

人/小鼠BDNF的最低可测剂量（MDD）一般小于1.552pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

此ELISA试剂盒经由R&D Systems生产的大肠杆菌表达的高纯度重组人/小鼠BDNF蛋白所校正。

E. 线性

在不同类型样本中掺入高浓度的人/小鼠BDNF，然后用稀释剂将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养上清（n=4)	人血清（n=5)	小鼠血清（n=5)
1:2	平均值/期待值（%）	92	96	93
	范围（%)	82 - 103	93 - 101	90 - 103
1:4	平均值/期待值（%）	105	98	100
	范围（%)	84 - 114	93 - 101	90 - 111
1:8	平均值/期待值（%）	101	110	107
	范围（%)	87 - 113	105 - 113	96 - 116
1:16	平均值/期待值（%）	101	110	106
	范围（%)	87 - 110	106 - 117	97 - 119

F. 样本预值

人血清样本 - 使用本试剂盒检测了5份人血清样本中BDNF的水平。5份样本的检测值在9125 to 20667.5pg/mL之间，平均值为15589.3pg/mL。

小鼠血清样本 - 使用本试剂盒检测了5份小鼠血清样本中BDNF的水平。5份样本的检测值均低于人/小鼠BDNF最低标准品，23.4pg/mL。

G. 特异性

将以下因子配置成50ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

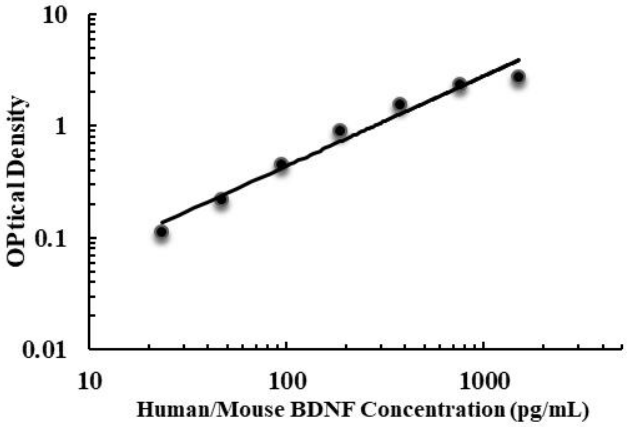
Recombinant human	
B-NGF	NT-3
GDNF	NT-4

重组人TrkB/Fc嵌合体在本试剂盒中没有交叉反应，但在浓度大于100pg/mL时有干扰作用。

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



pg/mL	OD	Average	Corrected
0	0.059 0.057	0.058	-
23.4	0.170 0.172	0.171	0.113
46.9	0.284 0.271	0.278	0.220
93.8	0.527 0.498	0.513	0.455
187.5	1.006 0.949	0.978	0.920
375	1.648 1.616	1.632	1.574
750	2.442 2.414	2.428	2.370
1500	2.827 2.836	2.832	2.774

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human/Mouse BDNF Microplate	包被小鼠抗人/小鼠 BDNF 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human/Mouse BDNF Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human/Mouse BDNF Detection antibody	生物素化的 BDNF 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent (10×)	浓缩的试剂稀释液（10×）	1 瓶
Calibrator Diluent (1×)	标准品稀释剂（1×）	2 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB 底物溶液	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP A</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释剂（1×）</td><td>2-8℃储存，最多 30 天*</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封：2-8℃储存，最多 30 天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450nm检测波长的吸收值及540nm或570nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 500mL量筒

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。使用后请彻底洗手。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于-20℃冰箱内，避免反复冻融。样本可能需要用标准品稀释剂（1×）稀释。

血清样本: 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000xg离心15分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。

B. 样本准备工作

细胞培养上清样本需要用标准品稀释剂（1×）2倍稀释后进行检测，例如：100μL样本+100μL标准品稀释剂（1×）。

人血清样本需要用标准品稀释剂（1×）100倍稀释后进行检测，例如：10μL人血清加到90μL标准品稀释剂（1×）中，充分混匀，即10倍稀释。然后取10μL 10倍稀释后的样本加到90μL标准品稀释剂（1×）中，充分混匀，即制备成100倍稀释的样本。

小鼠血清样本需要用标准品稀释剂（1×）2倍稀释后进行检测，例如：100μL样本+100μL标准品稀释剂（1×）。

C. 检测前准备工作

使用前请将所有试剂放置于室温

洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20mL浓缩洗涤液用蒸馏水或去离子水稀释配置成500mL工作浓度的洗涤液。未用完的放回4℃。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配置成试剂稀释液（1×）。

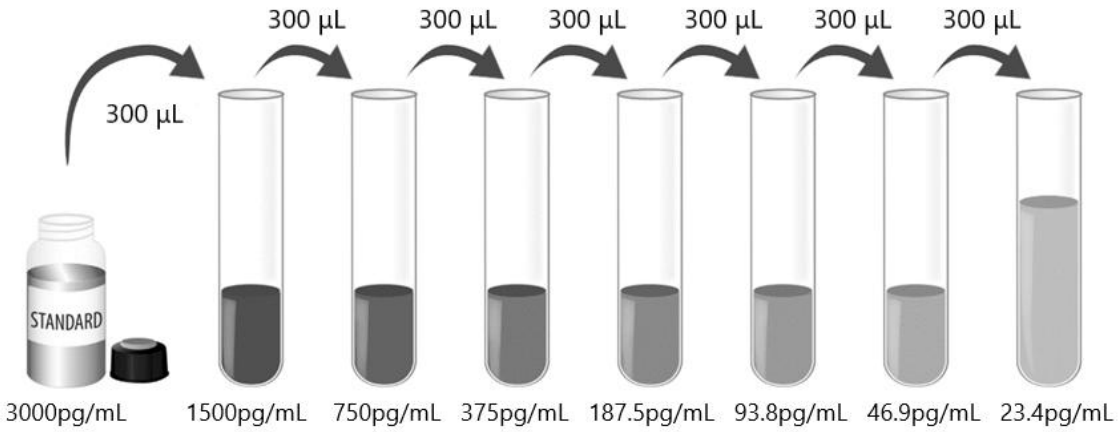
检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少15分钟，其充分溶解。如有需要分装保存。用试剂稀释液（1×）稀释至检测抗体（1×），至少在使用前15分钟准备。

链霉亲和素- HRP A: 开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

BDNF标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签，得到浓度为3000pg/mL标准品母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入300μL标准品稀释剂（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。1500pg/mL管作标准曲线最高点，标准品稀释剂（1×）可用作标准品零点（0pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配置不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板胶纸可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀，请充分混合。

VII.操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 分别将不同浓度标准品，实验样本或者质控品加入相应孔中，每孔100μL。用封板膜封住反应孔，室温孵育2小时。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；（样本需要稀释，详情参见样本制备部分。）
4. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400μL，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
5. 在每个微孔内加入100 μL配置好的检测抗体（1×）。用封板胶纸封住反应孔，室温孵育2小时；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100μL稀释好的链霉亲和素- HRP A工作液。用封板膜封住反应孔，室温孵育20分钟，**注意避光；**
8. 重复第4步洗板操作；
9. 在每个微孔内加入100μL TMB底物溶液，室温孵育20分钟，**注意避光**；
10. 在每个微孔内加入50μL终止液，请轻拍微孔板，使溶液混合均匀。孔内溶液颜色会从蓝色变为黄色；
11. 加入终止液后10分钟内，使用酶标仪测量450nm的吸光度值，设定540nm或570nm作为校正波长。如果没有使用双波长校正，结果准确度可能会受影响；
12. **计算结果：**将每个标准品和样品的校正吸光度值（OD450-OD540/OD570），复孔读数取平均值，然后减去平均零标准品OD值。使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一种方法是，可以通过绘制标准品浓度做对数与相应OD值对数生成曲线，并通过回归分析确定最佳拟合线。通过样本的OD值，可从标准曲线上得到样本中人/小鼠BDNF的浓度。如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置