Quantikine® ELISA

Total BDNF Immunoassay

Catalog Number DBNT00
SBNT00
PDBNT00

For the quantitative determination of free- and Trk-bound Brain-Derived Neurotrophic Factor (Total BDNF).

VALIDATED SAMPLE TYPES	HUMAN	MOUSE	RAT	CANINE	PORCINE
Cell culture supernates	√	√	√	√	√
Tissue Lysates	√	√	√	√	√
Serum	√	√	√	√	√
EDTA plasma				√	√
Heparin plasma				√	√
Platelet-poor EDTA plasma	√	√	√		
Platelet-poor heparin plasma	√	√	√		
Urine	√	√	√		
Human milk	√				

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Brain-Derived Neurotrophic Factor (BDNF) is a member of a small group of neurotrophins that regulate many aspects of neuronal development and function in both the central (CNS) and peripheral nervous systems. This family of secreted proteins also includes NGF, NT-3, and NT-4. BDNF is synthesized as a glycosylated and glycosulfated prepropeptide that is proteolytically processed to generate a non-glycosylated mature protein (1, 2). ProBDNF can be processed intracellularly by the serine proteases Furin, PCSK5, and PCSK6; however, the majority of proBDNF is secreted and cleaved in the extracellular environment by Plasmin and MMP-7 (1-6). Its transcription is controlled by nine different promoters. Alternative splicing of the different 5' un-translated exons to a common 3' exon, which encodes the BDNF protein, generates nine distinct transcripts that display differences in distribution, translation efficacy, and possibly function (6-8). The mature BDNF protein has a molecular weight of approximately 13 kDa. It exists as a noncovalently-linked homodimer in solution due to the formation of three disulfide bonds between six cysteine residues, which are conserved among all neurotrophins (2, 9-11). The amino acid sequences of the mature human, mouse, rat, canine, and porcine proteins are 100% identical. In fact, the primary structure of BDNF has been shown to be conserved among all mammalian species examined (2, 9).

BDNF is expressed both during development and through adulthood (12). It is the most widely expressed neurotrophin in the CNS with the highest levels of expression being detected in the hippocampus, amygdala, neocortex, and cerebellum (2, 5, 12, 13). It is also present in the spinal cord and fetal eye, and is expressed by both neurons and glial cells (13-16). In the nervous system, its expression and secretion are tightly controlled by neuronal activity and intracellular calcium levels (2, 6, 13). Additionally, pro-inflammatory cytokines and Progesterone have been shown to regulate its expression (8, 16). BDNF is also expressed outside the nervous system, in the placenta, heart, lung, and skeletal muscle, and by various cells types including fibroblasts, megakaryocytes/platelets, and smooth muscle cells (7, 17-22); however, the majority of circulating BDNF originates from the CNS. BDNF can cross the blood-brain barrier and studies have demonstrated a high positive correlation between serum BDNF levels and cortical and hippocampal BDNF expression (23). A common single nucleotide polymorphism (SNP) can occur in the pro-domain of BDNF that results in a substitution of a methionine for valine at codon 66 (Val66Met). This SNP impairs BDNF sorting into secretory granules and reduces activity-dependent secretion of BDNF (5, 6, 13).

BDNF is involved in a wide variety of activities. It regulates nearly all aspects of neural circuit development including neural stem cell survival and differentiation, axon/dendrite differentiation, synapse formation and maturation, and refinement of developing circuits (6). In the adult, BDNF has been shown to alter the efficacy of synaptic transmission at both glutamatergic and GABAergic synapses (6, 13). It also has emerged as a key regulator of synaptic plasticity and memory as research has shown that BDNF is needed for the induction and maintenance of late-phase long-term potentiation (2, 5, 6, 13, 24, 25), while the Val66Met SNP has been shown to impair episodic memory function (6, 13). BDNF is also believed to be critical for neuroprotection. Studies have shown that BDNF can rescue different types of neurons following various ischemic, traumatic, and toxic assaults (16, 26). These diverse biological functions of BDNF are mediated by the TrkB and NGF R/TNFRSF16 receptors (1, 5, 6, 13, 27). Additionally, it has been shown that secreted proBDNF can bind to NGF R/TNFRSF16 and induce long-term depression and cell apoptosis (5, 28). Abnormal BDNF levels, TrkB levels, and BDNF/TrkB signaling have been associated with a variety of conditions including depression, biopolar disorder, schizophrenia, epilepsy, Alzheimer's disease, Huntington's disease, and substance abuse (23, 25, 29-34). Thus, measuring total BDNF protein levels (i.e. both free BDNF and BDNF bound to TrkB) can provide insight into the mechanisms underlying these disorders.

The Quantikine® Total BDNF Immunoassay is a 3.5 hour solid phase ELISA designed to measure BDNF in cell culture supernates, tissue lysates, serum, plasma, urine, and human milk. It contains recombinant human BDNF expressed in *Sf* 21 cells and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant BDNF accurately. Results obtained measuring natural BDNF showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural BDNF.

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. An enzyme-linked monoclonal antibody specific for BDNF is added to the wells. Following a wash to remove any unbound antibody-enzyme 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.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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 diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution 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.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DBNT00	CATALOG # SBNT00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Total BDNF Microplate	894987	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for BDNF.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Total BDNF Standard	894989	2 vials	12 vials	Recombinant human BDNF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Total BDNF Conjugate	894988	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for BDNF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-123	896050	1 vial	6 vials	11 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5K	895119	2 vials	12 vials	21 mL/vial of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DBNT00 contains sufficient materials to run an ELISA on one 96 well plate.
SBNT00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDBNT00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Total BDNF Controls (optional; R&D Systems®, Catalog # QC218).

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- Lysis Buffer 17 (R&D Systems®, Catalog # 895943).
- PBS

or

- RIPA buffer with protease inhibitors

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Lysates - Tissue must be lysed prior to assay as directed in the Sample Values section.

Human 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.

Mouse/Rat Serum - Allow blood samples to clot for 2 hours at room temperature before centrifugation for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Canine Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 30 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Porcine Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE COLLECTION & STORAGE *CONTINUED*

Canine Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 30 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.

Porcine Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 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.

Note: *Canine and porcine platelet-poor samples were not available for testing.*

Platelet-poor Plasma - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly icteric samples are not suitable for use in this assay.

BDNF is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of BDNF, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical Laboratory and Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

Human Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Mouse/Rat Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional particulates that may appear after storage.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Human serum samples require a 100-fold dilution due to endogenous levels. A suggested 100-fold dilution can be achieved by adding 20 µL of sample to 180 µL of Calibrator Diluent RD5K. Complete the 100-fold dilution by adding 20 µL of the diluted sample to 180 µL Calibrator Diluent RD5K.

Rat serum samples require a 20-fold dilution due to endogenous levels. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5K.

Human platelet-poor plasma samples require a 5-fold dilution due to endogenous levels. A suggested 5-fold dilution is 30 µL of sample + 120 µL of Calibrator Diluent RD5K.

Mouse urine samples require a 2-fold dilution due to a matrix effect. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5K.

REAGENT PREPARATION

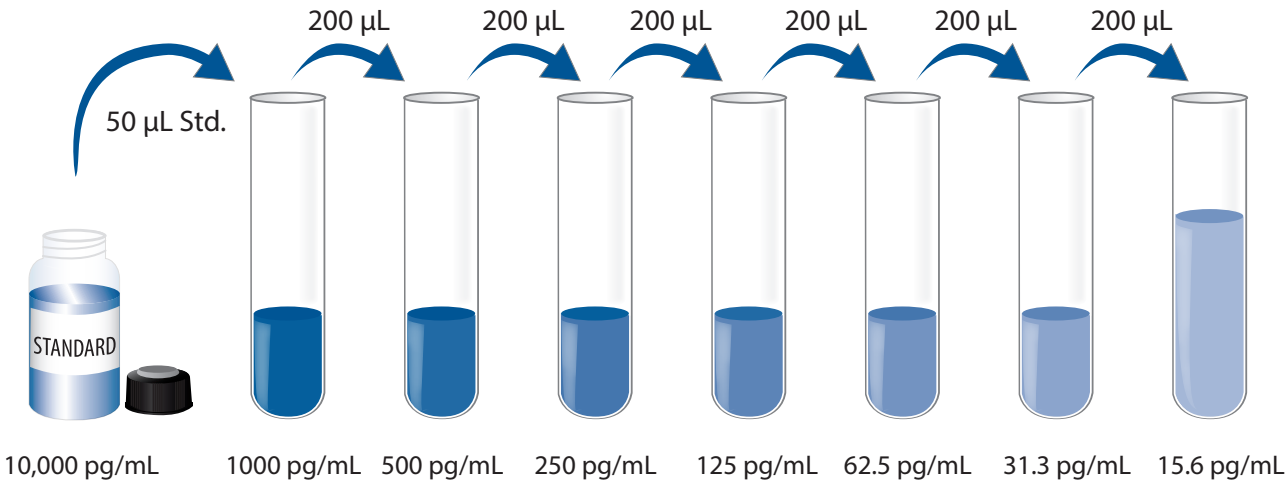
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate into 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Total BDNF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Total BDNF Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5K into the 1000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5K serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, and samples 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 50 µL of Assay Diluent RD1-123 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for **2 hours** at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (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.
6. Add 200 µL of Total BDNF Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

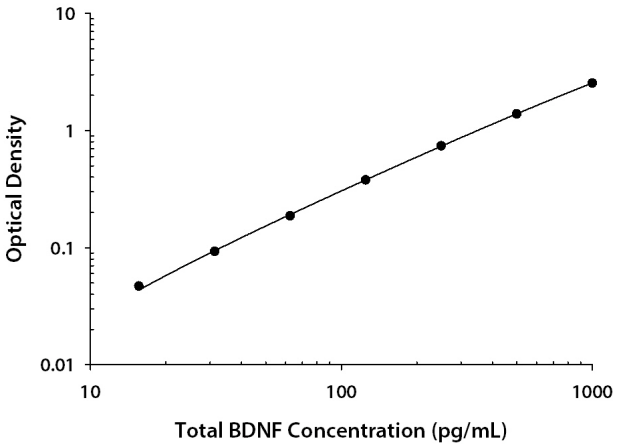
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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.

TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.007 0.009	0.008	—
15.6	0.053 0.056	0.055	0.047
31.3	0.100 0.101	0.101	0.093
62.5	0.191 0.198	0.195	0.187
125	0.383 0.391	0.387	0.379
250	0.741 0.753	0.747	0.739
500	1.366 1.423	1.395	1.387
1000	2.537 2.570	2.554	2.546

PRECISION

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

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	97.9	311	586	98.8	296	594
Standard deviation	3.12	7.52	17.5	7.07	12.7	28.0
CV (%)	3.2	2.4	3.0	7.2	4.3	4.7

SENSITIVITY

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of Total BDNF ranged from 0.372-1.35 pg/mL. The mean MDD was 0.997 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human BDNF produced at R&D Systems®.

The NIBSC/WHO 1st WHO Reference Reagent 96/534 recombinant human BDNF Standard was evaluated in this assay. The dose response curve of the NIBSC Reagent 96/534 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Total BDNF kit to approximate NIBSC Units, use the equation below.

NIBSC (96/534) approximate value (IU/mL) = 0.0006 x Quantikine® Total BDNF value (pg/mL)

RECOVERY

The recovery of Total 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)	103	97-113%
Lysis buffer (n=4)	94	87-100%

Human Samples	Average % Recovery	Range
Platelet-poor EDTA plasma (n=4)	95	76-110%
Platelet-poor heparin plasma (n=4)	95	75-111%
Urine (n=4)	93	75-106%
Human milk (n=4)	94	87-99%

Mouse Samples	Average % Recovery	Range
Serum (n=4)	103	95-111%
Platelet-poor EDTA plasma (n=4)	105	100-114%
Platelet-poor heparin plasma (n=4)	100	87-114%
Urine* (n=4)	100	92-110%

Rat Samples	Average % Recovery	Range
Serum* (n=4)	108	100-119%
Platelet-poor EDTA plasma* (n=4)	102	98-111%
Platelet-poor heparin plasma* (n=4)	105	96-114%
Urine (n=4)	93	88-110%

*Samples were diluted prior to assay.

Canine Samples	Average % Recovery	Range
Serum (n=4)	95	80-102%
EDTA plasma (n=4)	96	91-103%
Heparin plasma (n=4)	94	83-105%

Porcine Samples	Average % Recovery	Range
Serum (n=4)	100	93-107%
EDTA plasma (n=4)	96	89-107%
Heparin plasma (n=4)	100	90-110%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of Total BDNF were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

Human Samples		Cell culture supernates* (n=3)	Tissue lysates* (n=3)	Serum* (n=4)	Platelet-poor		Urine (n=4	Human milk (n=4)
					EDTA plasma* (n=4)	Heparin plasma* (n=4)		
1:2	Average % of Expected	101	103	102	99	106	101	104
	Range (%)	100-102	99-107	98-104	95-100	103-109	97-102	100-106
1:4	Average % of Expected	100	102	104	102	110	100	109
	Range (%)	99-101	98-110	99-110	99-104	107-111	93-105	104-116
1:8	Average % of Expected	101	99	107	106	111	96	111
	Range (%)	101-101	93-107	100-115	101-110	103-117	86-103	102-120
1:16	Average % of Expected	103	98	108	104	111	98	114
	Range (%)	101-105	90-104	101-117	98-111	104-116	88-111	104-124

Mouse Samples		Tissue lysates* (n=2)	Serum (n=4)	Platelet-poor		Urine (n=4)
				EDTA plasma (n=4)	Heparin plasma (n=4)	
1:2	Average % of Expected	107	100	101	102	104
	Range (%)	106-107	98-102	100-104	101-103	101-105
1:4	Average % of Expected	108	103	106	106	105
	Range (%)	106-109	102-106	101-110	104-109	103-106
1:8	Average % of Expected	106	109	111	107	104
	Range (%)	102-110	106-117	105-116	103-111	102-108
1:16	Average % of Expected	111	115	118	112	111
	Range (%)	110-113	106-124	112-125	106-118	102-125

Rat Samples		Serum* (n=4)	Platelet-poor		Urine (n=4)
			EDTA plasma (n=4)	Heparin plasma (n=4)	
1:2	Average % of Expected	99	99	101	99
	Range (%)	96-100	97-102	98-105	95-102
1:4	Average % of Expected	99	103	104	104
	Range (%)	97-100	99-109	99-113	99-110
1:8	Average % of Expected	100	107	104	105
	Range (%)	98-101	100-113	100-108	100-108
1:16	Average % of Expected	98	106	107	107
	Range (%)	95-102	99-116	106-108	106-111

*Samples were diluted prior to assay.

For research use only. Not for use in diagnostic procedures.

LINEARITY *CONTINUED*

Canine Samples		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	101	101	104
	Range (%)	99-103	93-105	102-108
1:4	Average % of Expected	106	105	108
	Range (%)	101-109	101-108	106-111
1:8	Average % of Expected	108	107	109
	Range (%)	101-115	105-109	104-113
1:16	Average % of Expected	111	108	111
	Range (%)	102-120	106-109	104-115

Porcine Samples		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	101	100	103
	Range (%)	99-105	94-103	99-106
1:4	Average % of Expected	103	105	103
	Range (%)	99-105	94-117	99-108
1:8	Average % of Expected	103	102	101
	Range (%)	102-107	93-105	96-105
1:16	Average % of Expected	104	105	102
	Range (%)	101-111	95-118	96-109

		Cell culture media (n=4)	Lysis buffer (n=4)
1:2	Average % of Expected	96	105
	Range (%)	89-100	99-111
1:4	Average % of Expected	94	108
	Range (%)	87-99	101-111
1:8	Average % of Expected	92	111
	Range (%)	86-95	104-115
1:16	Average % of Expected	87	112
	Range (%)	84-91	108-117

SAMPLE VALUES

Serum/Plasma/Urine/Human milk - Samples from apparently healthy volunteers were evaluated for the presence of Total BDNF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Human serum (n=36)	35,615	9042-57,830	9790
Human PP EDTA plasma (n=36)	307	94.9-902	197
Human PP heparin plasma (n=36)	595	100-2245	491
Rat serum (n=10)	5253	2554-6966	1438
Rat PP EDTA plasma (n=5)	294	87.5-416	138
Rat PP heparin plasma (n=5)	294	103-713	248

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Human urine (n=20)	ND	0	ND
Human milk (n=10)	ND	0	ND
Mouse serum (n=10)	55.4	50	ND-147
Mouse PP EDTA plasma (n=5)	21.4	60	ND-29.7
Mouse PP heparin plasma (n=5)	17.5	40	ND-19.1
Mouse urine (n=5)	ND	0	ND
Rat urine (n=5)	ND	0	ND
Canine serum (n=4)	ND	0	ND
Canine EDTA plasma (n=4)	ND	0	ND
Canine heparin plasma (n=4)	ND	0	ND
Porcine serum (n=4)	ND	0	ND
Porcine EDTA plasma (n=4)	ND	0	ND
Porcine heparin plasma (n=4)	ND	0	ND

ND=Non-detectable

SAMPLE VALUES *CONTINUED*

Cell Culture Supernates:
Human peripheral blood leukocytes (1 x 10^6 cells) were cultured in RPMI supplemented with 10% fetal bovine serum. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of Total BDNF.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	25.5	21.2
Stimulated	24.4	24.4

Human peripheral blood mononuclear cells (50 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate overnight. The cells were cultured unstimulated or stimulated with 1.0 µg/mL LPS for 1 day. Aliquots of the cell culture supernates were removed, assayed for levels of Total BDNF, and measured 2870 pg/mL and 2853 pg/mL, respectively.

MG-63 human osteosarcoma cells were cultured in DMEM supplemented with 5% fetal bovine serum and 4 mM L-glutamine and grown until confluent. An aliquot of the cell culture supernate was removed, assayed for Total BDNF, and measured 156 pg/mL.

Brain tissues from mice and rats were removed and rinsed in PBS and kept on ice. The tissue was homogenized using a tissue homogenizer and seeded into media containing RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated for 2 or 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of Total BDNF.

Sample Type	Observed Levels (pg/mL)
Mouse brain (2 days)	306
Rat brain (3 days)	196

Tissue lysates:
Human brain tissues were prepared in RIPA buffer with protease inhibitors. The working buffer was kept on ice and used within 2-3 days. Aliquots of the tissue lysates were removed and assayed for Total BDNF.

Sample Type	pg/mg of Cell Lysate
Human cerebellum	515
Human hypothalamus	441

Brains from mice were rinsed with PBS and homogenized with a tissue homogenizer in PBS. An equal volume of Lysis Buffer 17 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. An aliquot of each cell lysate was removed, assayed for levels of Total BDNF, and measured 1472 pg/mg of cell lysate.

SPECIFICITY

This assay recognizes natural and recombinant free, trk-bound, pro-, and mature BDNF.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a low level recombinant human BDNF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

BDNF (pro-domain, aa 19-128)
CNTF
β-NGF
NGF R
NT-3
NT-4
TrkA
TrkB
TrkC

Recombinant mouse:

BDNF (pro-domain, aa 19-130)
TrkB
TrkC
β-NGF
NGF R
NT-4

Recombinant rat:

β-NGF

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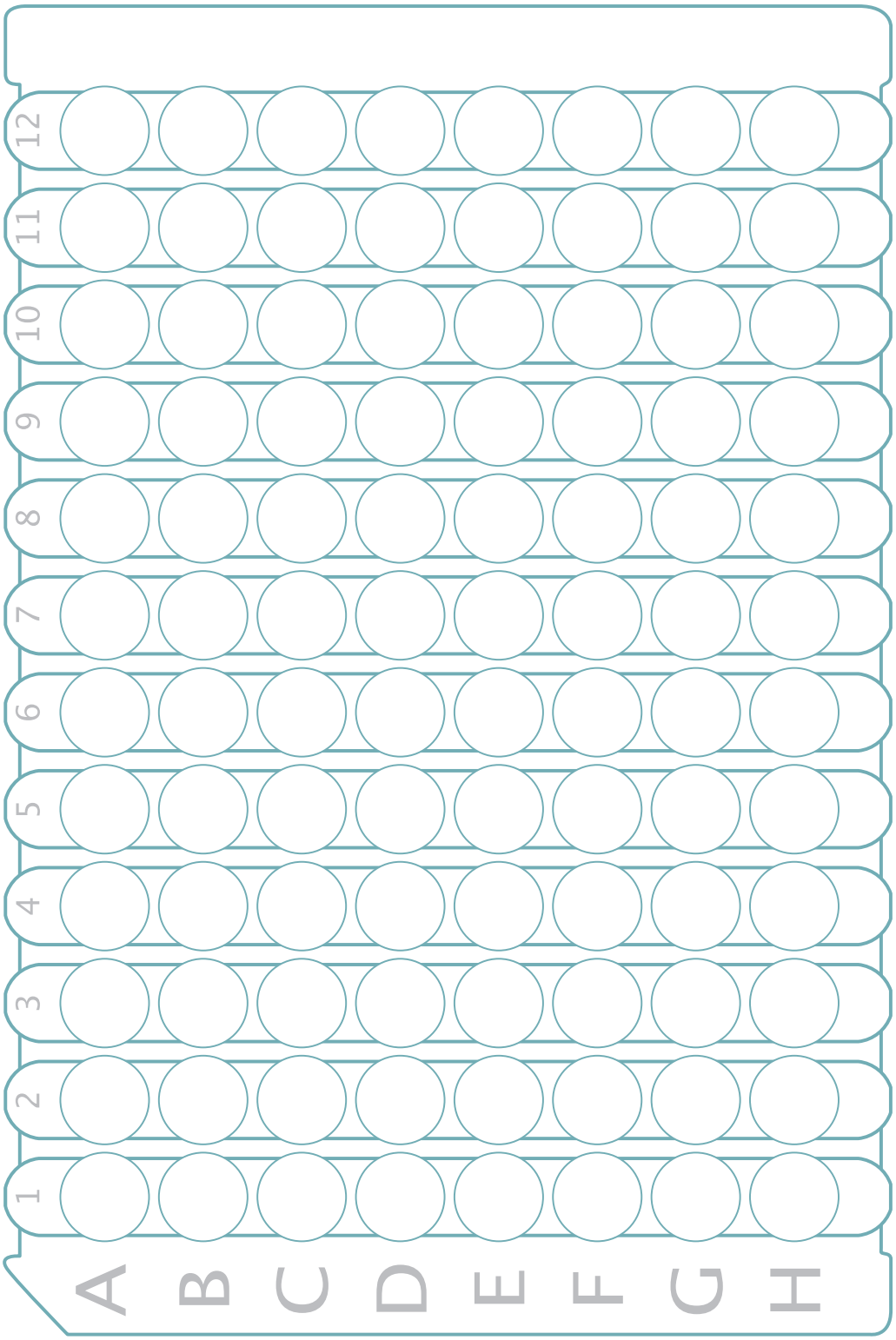
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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