ab229395 – Human BDNF CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of free BDNF in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, and CSF.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab229395

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except Typical Standard Curve and Sensitivity, were collected using the colorimetric version of this kit (ab212166).

Materials Supplied

Item	Quantity	Storage Condition
Human BDNF Capture Antibody 10X	600 µL	+4°C
Human BDNF Detector Antibody 10X	600 µL	+4°C
Human BDNF Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI	6 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H_2O_2, 3%)	50 µL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI. Mix thoroughly and gently.

CatchPoint HRP Development Solution: Just prior to use prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer. For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 µL 100X Stoplight Red Substrate and 12 µL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. Mix thoroughly and gently

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the BDNF standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 2,000 pg/mL **Stock Standard** Solution.
2. Label 11 tubes, Standards 2– 12. The **Stock Standard** will be standard #1.
3. Add 150 µL of Sample Diluent NS into tube number 2-12.
4. Use the Stock Standard to prepare the following dilution series. Standard #12 contains no protein and is the Blank control.

   Standards will be added to the plate according to **Assay Procedure** step 3. If desired all 12 standards can be used for a full standard curve. Alternatively, to commit fewer wells to the standard curve, select a subset of at least 7 standards plus the blank control. If 7 standards are desired, we recommend standards #2-8.

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	150	0		2,000
2	Standard#1	150	150	2,000	1,000
3	Standard#2	150	150	1,000	500
4	Standard#3	150	150	500	250
5	Standard#4	150	150	250	125
6	Standard#5	150	150	125	62.5
7	Standard#6	150	150	62.5	31.3
8	Standard#7	150	150	31.3	15.6
9	Standard#8	150	150	15.6	7.8
10	Standard#9	150	150	7.8	3.9
11	Standard#10	150	150	3.9	2.0
12	None	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Human Serum	1% - 10%
Human Plasma - Heparin	1% - 10%
Human Plasma - EDTA	1% - 10%
Human Plasma - Citrate	1% - 10%
Rat Serum	1.25% - 20%
L929 Stimulated Supernatant*	≤ 50%
Human Cerebrospinal Fluid*	≤ 50%

*Based on spiked sample

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:10 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.
Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:10 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.
Cerebrospinal Fluid (CSF) Dilute cerebrospinal fluid at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples 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, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.
8. Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at www.softmaxpro.org.
9. Analyze the data as described below.

Mode	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of free human BDNF.

The standard protein in this kit is mature full length human BDNF.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.

Spiked protein experiments were used to validate cell culture supernatant and CSF sample types.

Milk, saliva, urine, cell extract, and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY
50 ng/mL and 500 pg/mL of recombinant human TRKB was tested for cross reactivity. No cross reactivity was observed.

Cross reactivity against the precursor form of BDNF has not been tested.

INTERFERENCE
50 ng/mL and 1 ng/mL of recombinant human TRKB was tested for interference with 200 pg/mL of recombinant human BDNF. 50 ng/mL of recombinant human TRKB decreased interpolated value of 200 pg/mL of recombinant human BDNF by 76%. 1 ng/mL of recombinant human TRKB decreased interpolated value of 200 pg/mL of recombinant human BDNF by 39%.

SPECIES REACTIVITY
This kit recognizes human and rat free BDNF protein.

Other species reactivity was determined by measuring 10% serum samples of various species, interpolating the BDNF protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the BDNF protein concentration in human serum assayed at the same dilution.

7% cross-reactivity was observed in pooled normal bovine and mouse serum. 226% cross-reactivity was observed in pooled rat serum.

Other species reactivity not determined.

CALCULATION
- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices
- Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.
- Create a standard curve by plotting the average blank control subtracted fluorescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most fluorescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted fluorescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA
Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

Standard Curve Measurements			
Concentration (pg/mL)	RFU		Mean RFU
	1	2	
0	53	55	54
2.0	88	82	85
3.9	107	125	116
7.8	143	169	156
15.6	246	257	252
31.3	507	520	513
62.5	1,180	1,363	1,271
125	2,192	2,308	2,250
250	4,520	4,629	4,574
500	8,038	8,001	8,019
1,000	14,703	14,839	14,771
2,000	22,936	22,505	22,720

Table 1. Example of human BDNF standard curve in Sample Diluent NS. The BDNF standard curve was prepared as described in the Standard Preparation section. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 1.5 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=18) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of BDNF were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
10% Human Serum	97	94 - 104
5% Human Plasma - Citrate	103	95 - 111
25% Human Plasma - EDTA	98	85 - 108
5% Human Plasma - Heparin	103	96 - 112
2.5% Rat Serum	107	103 - 110
50% Cell Culture Supernatant	106	103 - 109
50% Human Cerebrospinal Fluid	95	88 - 104

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native BDNF was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	10% Human Serum	10% Human Plasma (Citrate)	10% Human Plasma (EDTA)	10% Human Plasma (Heparin)	20% Rat Serum
Undiluted	pg/mL	93.5	207.6	70.2	90.4	423.0
	% Expected value	100	100	100	100	100
2	pg/mL	51.0	104.4	35.2	41.3	238.3
	% Expected value	109	101	100	91	113
4	pg/mL	24.4	56.0	17.1	21.5	123.2
	% Expected value	104	108	97	95	117
8	pg/mL	11.1	29.4	8.4	10.4	59.9
	% Expected value	95	113	96	92	113
16	pg/mL	ND	15.3	ND	ND	30.4
	% Expected value	ND	118	ND	ND	115

ND – Not Detectable

Recombinant BDNF was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Cell Culture Media	50% L929 Stimulated Supernatant	50% Human CSF
Undiluted	pg/mL	529.2	573.4	498.3
	% Expected value	100	100	100
2	pg/mL	248.8	247.9	265.5
	% Expected value	94	86	107
4	pg/mL	128.3	128.8	132.3
	% Expected value	97	90	106
8	pg/mL	63.5	61.6	65.1
	% Expected value	96	86	104
16	pg/mL	32.9	32.2	28.1
	% Expected value	99	90	90

Precision

Mean coefficient of variations of interpolated values of BDNF from three concentrations of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	2.8	5.3

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)