

ab324919 – Free BDNF SimpleStep ELISA® Kit – Extracellular

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

For overview, typical data and additional information please visit: www.abcam.com/ab324919

This kit is available in a 384-well plate format. This plate utilizes smaller volumes of standards and samples per well. Directions for using this format can be found on pages 6-7.

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.

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Free BDNF Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Free BDNF Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Free BDNF Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent CPI2	6 mL	10 x 6 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
TMB Development Solution	12 mL	120 mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	10 x 96 wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

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

Microplate reader capable of measuring absorbance at 450 or 600 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 100 mL 1X Wash Buffer PT combine 10 mL Wash Buffer PT 10X with 90 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. 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 CPI2. 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 Free 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 3.84 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1–8.
3. Add 290 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock Standard	30	290	3,840	360
2	Standard#1	150	150	360	180
3	Standard#2	150	150	180	90
4	Standard#3	150	150	90	45
5	Standard#4	150	150	45	22.5
6	Standard#5	150	150	22.5	11.25
7	Standard#6	150	150	11.25	5.63
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Human Serum	0.31 - 5%
Human Plasma (Citrate)	0.63 - 10%
Human Plasma (EDTA)	0.78 - 6.25%
Human Plasma (Heparin)	1.56 - 12.5%
Stimulated L929 Supernatant	6.25 - 50%
Human Urine	≤ 25%
Human Cerebrospinal Fluid	≤ 50%
Mouse Serum	≤ 50%
Rat Serum	0.78 - 12.5%

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 1:20 for human samples, 1:2 for mouse samples, or 1:8 for rat samples 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 for plasma citrate samples, 1:16 for plasma EDTA samples, or 1:8 for plasma heparin samples 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. 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.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:4 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Cerebrospinal Fluid (CSF) 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.

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, resealed 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 TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.

Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

ASSAY SPECIFICITY

The antibodies in this kit were raised against rat pro-BDNF and additionally detect mature BDNF. Mature BDNF is 100% conserved among human, mouse, and rat. This kit recognizes free BDNF; human TrkB interfered at all concentrations tested (see interference section below).

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

Spiked protein experiments were used to validate mouse serum, human urine, and human CSF sample.

Saliva and milk samples have not been tested with this kit.

CROSS REACTIVITY

50 ng/mL of recombinant human beta-NGF was tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE

Recombinant rat pro-BDNF was assayed at 90 pg/mL in the presence of three different concentrations of recombinant human NGF R. The % decrease in BDNF signal is shown below.

Recombinant Human NGF R (pg/mL)	% Decrease in BDNF Signal
50,000	25%
1,000	15%
250	18%

Recombinant mature BDNF was assayed at 250 pg/mL in presence of four different concentrations of recombinant human TrkB. The % decrease in BDNF signal is shown below.

Recombinant Human TrkB (pg/mL)	% Decrease in BDNF Signal
50,000	86%
10,000	79%
1,000	30%
250	9%

SPECIES REACTIVITY

Human, mouse, and rat biologicals have been validated with this kit. Mature BDNF is 100% conserved among human, mouse, rat, cow, monkey, rabbit, dog, and pig. The kit is predicted to react with these additional species.

Cell culture media containing 10% fetal bovine serum (FBS) was tested. No detectable signal was observed.

Other species reactivity not determined.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant mature BDNF protein. The NIBSC/W unclassified purified 1st WHO reference reagent brain-derived neurotropic factor (BDNF) preparation 96/534 was evaluated in this kit.

The dose response curve of the unclassified standard BDNF parallels the SimpleStep standard curve.

To convert sample values obtained with the SimpleStep Free BDNF kit to approximate NIBSC 96/534 units, use the equation below.

NIBSC (96/534) approximate value (units/mL) = 0.00671 x SimpleStep Free BDNF value (pg/mL).

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance 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 microplate 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 absorbance 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 absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance 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)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.046	0.048	0.047
5.63	0.106	0.105	0.105
11.25	0.169	0.164	0.167
22.5	0.295	0.284	0.289
45	0.522	0.529	0.526
90	0.991	0.937	0.964
180	1.822	1.777	1.800
360	3.414	3.245	3.329

Table 1. Example of Free BDNF standard curve in Sample Diluent NS. The Free BDNF standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 1.75 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

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

Sample Type	Average % Recovery	Range (%)
1% Human Serum	102	99 - 109
5% Human Plasma (Citrate)	81	78 - 83
5% Human Plasma (EDTA)	106	98 - 114
5% Human Plasma (Heparin)	92	88 - 95
25% Stimulated L929 Supernatant	104	101 - 111
25% Human Urine	80	78 - 83
50% Human Cerebrospinal Fluid	100	95 - 105
50% Mouse Serum	89	83 - 93
2.5% Rat Serum	99	96 - 102

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 Free 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	5% Human Serum	10% Human Plasma (Citrate)	6.25% Human Plasma (EDTA)	12.5% Human Plasma (Heparin)
Undiluted	pg/mL	198.8	74.3	53.5	39.5
	% Expected value	100	100	100	100
2	pg/mL	100.7	36.6	30.0	22.8
	% Expected value	101	98	112	116
4	pg/mL	50.9	19.4	14.4	11.1
	% Expected value	102	104	108	112
8	pg/mL	27.1	10.7	7.4	5.0
	% Expected value	109	115	110	101
16	pg/mL	13.4	5.4	ND	ND
	% Expected value	108	117	ND	ND

ND – Not Detected – below product dynamic range

Native Free 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	50% Stimulated L929 Supernatant	12.5% Rat Serum
Undiluted	pg/mL	123.8	159.8
	% Expected value	100	100
2	pg/mL	67.3	83.6
	% Expected value	109	105
4	pg/mL	33.4	41.5
	% Expected value	108	104
8	pg/mL	17.5	21.9
	% Expected value	113	110
16	pg/mL	NL	11.1
	% Expected value	NL	111

NL – Non-Linear

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

Dilution Factor	Interpolated value	25% Human Urine	50% Human CSF	50% Mouse Serum
Undiluted	pg/mL	65.4	146.0	160.3
	% Expected value	100	100	100
2	pg/mL	34.7	81.2	81.1
	% Expected value	106	111	101
4	pg/mL	18.8	38.7	46.5
	% Expected value	115	106	116
8	pg/mL	9.6	21.5	23.5
	% Expected value	117	118	117
16	pg/mL	4.5	10.4	11.3
	% Expected value	111	114	113

Precision

Mean coefficient of variations of interpolated values of Free BDNF from a single concentration of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	3.3	2.5

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

DIRECTIONS FOR 384-WELL PLATE FORMAT:

Materials Supplied for 384-well Format

Item	Quantity	Storage Condition
Free BDNF Capture Antibody 10X	600 μ L	+4°C
Free BDNF Detector Antibody 10X	600 μ L	+4°C
Free BDNF Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	2 x 12 mL	+4°C
Stop Solution	2 x 12 mL	+4°C
SimpleStep Pre-Coated 384-Well Microplate	384 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:

Microplate reader capable of measuring absorbance at 450 or 600 nm in a 384-well plate.

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).

Optional: Automated liquid handler.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for one full plate. The sample volumes below are sufficient for running all 384 wells; adjust volumes as needed for the number of samples and dilution scheme for 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 200 mL 1X Wash Buffer PT combine 20 mL Wash Buffer PT 10X with 180 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 6 mL of the Antibody Cocktail combine 600 μ L 10X Capture Antibody and 600 μ L 10X Detector Antibody with 4.8 mL Antibody Diluent CPI2. 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 Free 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 3.84 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 290 μ L of Sample Diluent NS into tube number 1 and 75 μ L of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (μ L)	Volume of Diluent (μ L)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock Standard	30	290	3,840	360
2	Standard#1	75	75	360	180
3	Standard#2	75	75	180	90
4	Standard#3	75	75	90	45
5	Standard#4	75	75	45	22.5
6	Standard#5	75	75	22.5	11.25
7	Standard#6	75	75	11.25	5.63
8	Blank Control	0	75	0	0

Plate Preparation

The 384-well plate included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

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 for 384-well Plate Format

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. Add 12.5 μL of all sample or standard to appropriate wells.
3. Add 12.5 μL of the Antibody Cocktail to each well.
4. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 700 rpm.
5. Wash each well with 3 x 100 μL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 100 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.
6. Add 25 μL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 700 rpm.
Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.₆₀₀ equal to 1.0.
7. Add 25 μL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading. Proper mixing of the Stop Solution is required for proper measurement.
8. Alternative to 6 – 7: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec – 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 25 μL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus