ab326312 – Human beta Nerve Growth Factor SimpleStep ELISA® Kit (beta NGF), Chemiluminescent

For the quantitative measurement of beta Nerve Growth Factor in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, and urine.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326312

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 (ab193760).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human beta Nerve Growth Factor Capture Antibody 10X	600 µL	+4°C
Human beta Nerve Growth Factor Detector Antibody 10X	600 µL	+4°C
Human beta Nerve Growth Factor Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent 50BS	20 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+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:

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
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.

Sample Diluent 50BS may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

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.

Sample Diluent 25BS: Prepare Sample Diluent 25BS from Sample Diluent 50BS by adding one part Sample Diluent 50BS to one part Sample Diluent NS. To generate 12mL of Sample Diluent 25BS, combine 6mL of Sample Diluent 50BS with 6mL of Sample Diluent NS. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. 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 4BI. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

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).

For **cell culture supernatant or urine samples:**

1. Reconstitute the standard 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 3,500 pg/mL **Stock Standard #1** solution.
2. Label seven tubes, Standards 1– 7.
3. Add 150 µL of **Sample Diluent NS** into numbers 2-7.
4. Use the **Stock Standard #1** to prepare the following dilution series. Standard #7 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 #1**	225	0	3,500	3,500
2	**Stock Standard #1**	75	150	3,500	1,166.67
3	Standard#1	75	150	1,166.67	388.89
4	Standard#2	75	150	388.89	129.63
5	Standard#3	75	150	129.63	43.21
6	Standard#4	75	150	43.21	14.40
7	Blank Control	0	150	0	0

For **serum, plasma (citrate), or plasma (EDTA) samples:**

1. Reconstitute the standard by adding the volume of **Sample Diluent 50BS** indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 3,500 pg/mL **Stock Standard #1** solution.
2. Label eight tubes, Standards 1– 8.
3. Add 150 µL of **Sample Diluent 50BS** into tubes 2-8.
4. Use the **Stock Standard #1** 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 #1**	225	0	3,500	3,500
2	**Stock Standard #1**	75	150	3,500	1,166.67
3	Standard#1	75	150	1,166.67	388.89
4	Standard#2	75	150	388.89	129.63
5	Standard#3	75	150	129.63	43.21
6	Standard#4	75	150	43.21	14.40
7	Standard#5	75	150	14.40	4.80
8	Blank Control	0	150	0	0

For **plasma (heparin) samples:**

1. Reconstitute the standard by adding **Sample Diluent 25BS** indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 3,500 pg/mL **Stock Standard #1** solution.
2. Label eight tubes, Standards 1– 8.
3. Add 150µL of **Sample Diluent 25BS** into tubes 2-8.
4. Use the **Stock Standard #1** 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 #1**	225	0	3,500	3,500
2	**Stock Standard #1**	75	150	3,500	1,166.67
3	Standard#1	75	150	1,166.67	388.89
4	Standard#2	75	150	388.89	129.63
5	Standard#3	75	150	129.63	43.21
6	Standard#4	75	150	43.21	14.40
7	Standard#5	75	150	14.40	4.80
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	0.78 - 12.5%
Plasma – Citrate*	0.78 - 12.5%
Plasma – EDTA*	1.56 - 25%
Plasma – Heparin*	1.56 - 25%
Urine*	6.25 - 100%
PBMC Cell Culture Supernatant*	1.56 - 25%

*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 into Sample Diluent 50BS 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 into Sample Diluent 50BS (for plasma – citrate or plasm – EDTA) or Sample Diluent 25BS (for plasma – heparin) and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. 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. Store undiluted samples at -20°C or below. Avoid repeated freeze-thaw cycles. Dilute samples into Sample Diluent NS and assay.

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 30 minutes at room temperature on a plate shaker set to 750 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 30 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 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

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:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

Version 1 | 2026-03-24

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of human beta Nerve Growth Factor.

The standard protein in this kit is mature, full-length human beta Nerve Growth Factor.

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, and urine sample types.

25% serum from ten individual healthy human female/male donors was measured in duplicate. All values were below the detectable range of the assay.

Saliva, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY
50 ng/mL of recombinant human CNTF was tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE
50 ng/mL of recombinant human CNTF was tested for interference with 875 pg/mL of recombinant beta NGF. No interference was observed.

SPECIES REACTIVITY
No signal was observed in 25% serum from the following species: Mouse, Rat, Hamster, Rabbit, Dog, Goat, Pig, Cow.

CALCULATION
- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence 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 chemiluminescence 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 chemiluminescence 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 chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence 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)	RLU		Mean RLU
	1	2	
0	24,965	24,965	24,965
14.40	32,795	36,190	34,493
43.21	44,840	46,020	45,430
129.63	125,820	123,630	124,725
388.89	352,760	356,970	354,865
1,166.67	1,159,900	1,148,600	1,154,250
3,500	3,379,400	3,226,000	3,302,700

Table 1. Example of human beta Nerve Growth Factor standard curve in Sample Diluent NS. The beta Nerve Growth Factor standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	9,297	9,297	9,297
4.80	14,916	13,446	14,181
14.40	18,126	17,491	17,809
43.21	36,217	35,186	35,702
129.63	102,250	104,490	103,370
388.89	264,700	295,820	280,260
1,166.67	714,190	805,450	759,820
3,500	2,423,500	2,339,800	2,381,650

Table 2. Example of human beta Nerve Growth Factor standard curve in Sample Diluent 25BS. The beta Nerve Growth Factor standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	6,353	6,353	6,353
4.80	10,828	10,579	10,704
14.40	13,875	14,076	13,976
43.21	27,437	28,194	27,816
129.63	76,028	75,724	75,876
388.89	238,820	223,980	231,400
1,166.67	681,780	667,550	674,665
3,500	1,919,200	1,927,800	1,923,500

Table 3. Example of human beta Nerve Growth Factor standard curve in Sample Diluent 50BS. The beta Nerve Growth Factor standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent NS	16	10.3 pg/mL
Sample Diluent 25BS	16	4.0 pg/mL
Sample Diluent 50BS	16	1.2 pg/mL

Recovery

Three concentrations of beta Nerve Growth Factor 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 (%)
12.5% Human Serum	94	92 – 95
12.5% Human Plasma – Citrate	82	76 – 88
25% Human Plasma – EDTA	88	83 – 92
25% Human Plasma – Heparin	93	92 – 95
25 % PBMC supernatant	96	95 – 99
50% Human Urine	96	94 – 100

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.

Purified beta Nerve Growth Factor was spiked into the following biological samples and diluted in a 2-fold dilution series. Serum and plasma (citrate or EDTA) were diluted in Sample Diluent 50BS. Plasma (heparin) was diluted in Sample Diluent 25BS.

Dilution Factor	Interpolated value	12.5% Human Serum	12.5% Human Plasma (Citrate)	25% Human Plasma (EDTA)	25% Human Plasma (Heparin)
Undiluted	pg/mL	777	732	755	791
	% Expected value	**100**	**100**	**100**	**100**
2	pg/mL	429	379	403	419
	% Expected value	**110**	**103**	**107**	**106**
4	pg/mL	223	202	192	210
	% Expected value	**115**	**110**	**102**	**106**
8	pg/mL	110	106	88	107
	% Expected value	**113**	**116**	**93**	**108**
16	pg/mL	54	56	44	54
	% Expected value	**111**	**122**	**94**	**108**

Purified beta Nerve Growth Factor was spiked into the following biological samples and diluted in a 2-fold dilution series. Cell culture supernatant and urine samples were diluted in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Urine	25% PBMC Supernatant
Undiluted	pg/mL	377	259
	% Expected value	**100**	**100**
2	pg/mL	214	136
	% Expected value	**114**	**105**
4	pg/mL	106	77
	% Expected value	**113**	**118**
8	pg/mL	56	37
	% Expected value	**119**	**114**
16	pg/mL	27	17
	% Expected value	**113**	**105**

Precision

Mean coefficient of variations of interpolated values of from three concentrations of beta NGF within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	3.4	6.7

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

Version 1 | 2026-03-24

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