ab193760 – beta Nerve Growth Factor (beta NGF) Human SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of beta Nerve Growth Factor (beta NGF) in human serum, plasma, urine and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

Version 3a Last Updated 19 July 2023

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1. BACKGROUND

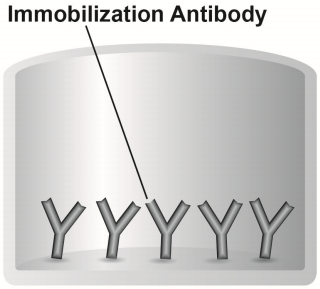
Beta Nerve Growth Factor (beta NGF) *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of beta Nerve Growth Factor protein in human serum, plasma, urine and cell culture supernatants.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

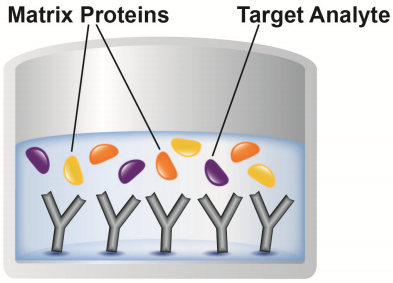
Beta Nerve Growth Factor is involved in the development of maintenance of the sympathetic and sensory nervous systems. As a ligand for NTRK1 and NGFR receptors, it's involved in cell signaling cascades that regulate neuronal proliferation, differentiation, and survival.

Beta Nerve Growth Factor is also involved in modulation of the immune system and in the trauma response, and has been implicated in a variety of responses including memory and mental health.

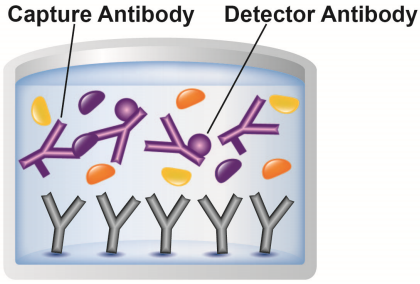
2. ASSAY SUMMARY



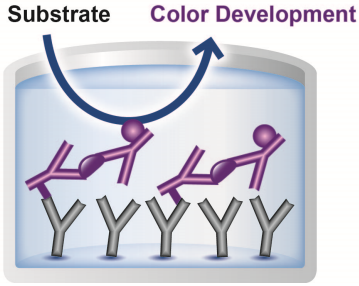
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Development Solution to each well and incubate. Add Stop Solution at a defined endpoint.

Alternatively, record color development kinetically after TMB substrate addition.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X beta NGF Capture Antibody	1 x 600 µL	+2-8ºC
10X beta NGF Detector Antibody	1 x 600 µL	+2-8ºC
beta NGF Human Lyophilized Recombinant Protein	2 Vials	+2-8ºC
Antibody Diluent 4BI	1 x 6 mL	+2-8ºC
10X Wash Buffer PT	20 mL	+2-8ºC
TMB Development Solution	12 mL	+2-8ºC
Stop Solution	12 mL	+2-8ºC
Sample Diluent NS	50 mL	+2-8ºC
Sample Diluent 50BS	20 mL	+2-8ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8ºC
Plate Seal	1	+2-8ºC

6. 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

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers
- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background

- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11)
- All samples should be mixed thoroughly and gently
- Avoid multiple freeze/thaw of samples
- Incubate ELISA plates on a plate shaker during all incubation steps
- When generating positive control samples, it is advisable to change pipette tips after each step
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. 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.

9.1 1X Wash Buffer PT

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

9.2 Sample Diluent 25BS (For normal human serum and plasma samples only)

Prepare Sample Diluent 25BS from Sample Diluent 50BS by diluting Sample Diluent 50BS 1:1 in Sample Diluent NS. To generate 12 mL Sample Diluent 25BS combine 6 mL of Sample Diluent 50BS with 6 mL Sample Diluent NS. Mix thoroughly and gently.

9.3 Antibody Cocktail

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

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following table describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the beta NGF standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the beta NGF standard by adding 500 µL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 3.5 ng/mL **Stock Standard** Solution.

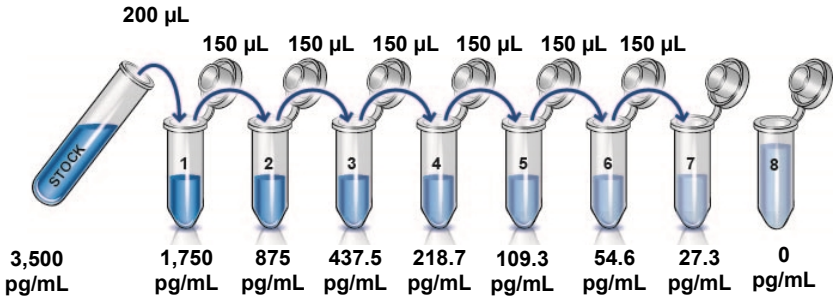
10.2 Label eight tubes, Standards 1– 8.

10.3 For **cell culture supernatant or urine samples** reconstitute the standard by adding Sample Diluent NS. Add 200 µL Sample Diluent NS into tube number 1 and 150 µL Sample Diluent NS into tube numbers 2-8.

For **serum, plasma –citrate, or plasma–EDTA samples** reconstitute the standard by adding Sample Diluent 50BS. Add 200 µL Sample Diluent 50BS into tube number 1 and 150 µL Sample Diluent 50BS into tube numbers 2-8.

For **plasma– heparin samples** reconstitute the standard by adding Sample Diluent 25BS. Add 200 µL Sample Diluent 25BS into tube number 1 and 150 µL Sample Diluent 25BS into tube numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
Human Serum	1:8 - 1: 128
Human Plasma - Citrate	1:8 - 1: 128
Human Plasma - EDTA	1:4 - 1:64
Human Plasma - Heparin	1:4 - 1:64
Human urine	1:1- 1: 16
PBMC supernatant	1:4-1: 64

11.1 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 plasma–EDTA) or Sample Diluent 25BS (for plasma–heparin) and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 **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 -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 **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.

12. 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

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 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. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 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.

13.8 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.

Alternative to 13.7 – 13.8: 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.

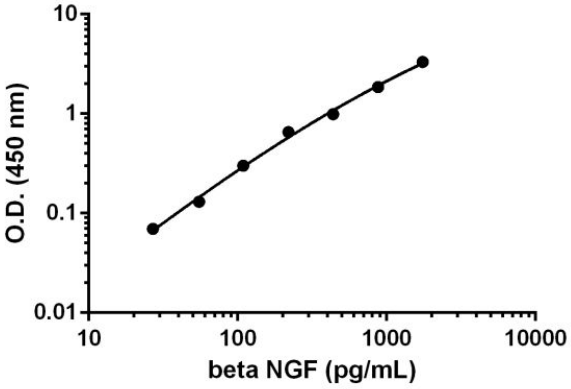
13.9 Analyze the data as described below.

14. CALCULATIONS

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

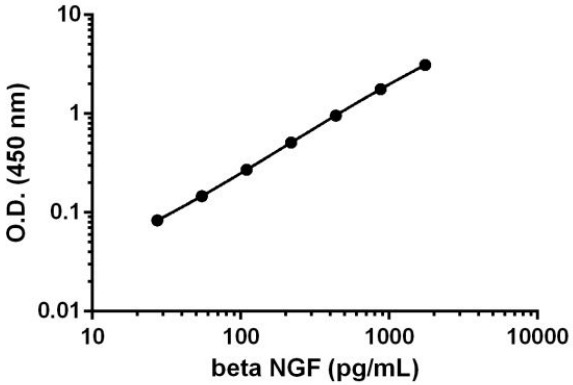
15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



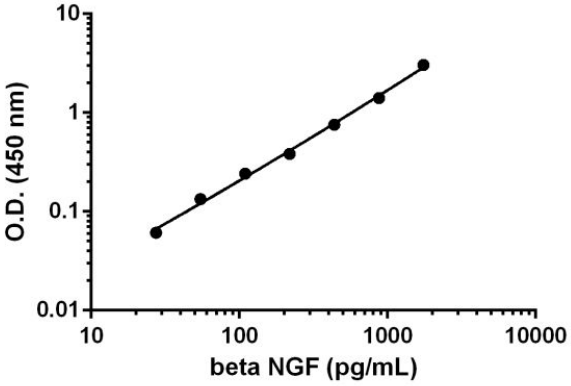
Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.067	0.071	0.069
27	0.144	0.134	0.139
55	0.195	0.205	0.200
109	0.359	0.383	0.371
219	0.734	0.712	0.723
438	1.039	1.083	1.061
875	1.894	1.961	1.928
1,750	3.323	3.456	3.389

Figure 1. Example of beta Nerve Growth Factor standard curve in Sample Diluent NS. The beta Nerve Growth Factor standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.074	0.072	0.073
27	0.080	0.086	0.083
55	0.132	0.155	0.146
109	0.255	0.287	0.271
219	0.476	0.547	0.512
438	0.900	1.016	0.960
875	1.654	1.893	1.773
1,750	2.995	3.231	3.113

Figure 2. Example of beta Nerve Growth Factor standard curve in Sample Diluent 25BS. The beta Nerve Growth Factor standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.075	0.076	0.076
27	0.139	0.142	0.140
55	0.218	0.208	0.213
109	0.313	0.331	0.322
219	0.443	0.485	0.464
438	0.814	0.854	0.834
875	1.461	1.517	1.489
1,750	3.069	3.187	3.128

Figure 3. Example of beta Nerve Growth Factor standard curve in Sample Diluent 50BS. The beta Nerve Growth Factor standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentrations.

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	24	10 pg/mL
Sample Diluent 25BS	24	10 pg/mL
Sample Diluent 50BS	25	6 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 –

Purified beta Nerve Growth Factor was spiked into the following biological samples and diluted in a 2-fold dilution series. Serum and Citrate or EDTA plasma were diluted in Sample Diluent 50BS. Heparin

plasma was diluted in Sample Diluent 25BS and media or urine samples were diluted in Sample Diluent NS.

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.

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	**100**	**100**	**100**	**100**
2	pg/mL	429	379	403	419
	% Expected	**110**	**103**	**107**	**106**
4	pg/mL	223	202	192	210
	% Expected	**115**	**110**	**102**	**106**
8	pg/mL	110	106	88	107
	% Expected	**113**	**116**	**93**	**108**
16	pg/mL	54	56	44	54
	% Expected	**111**	**122**	**94**	**108**

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

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of beta NGF within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	5	3
CV (%)	3.4	6.7

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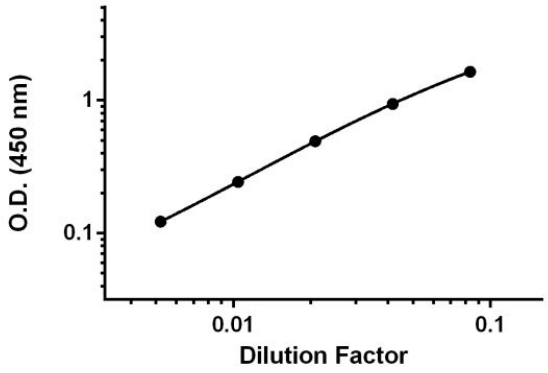

Figure 4. Titration of human serum (spiked with 875 pg/mL purified beta Nerve Growth Factor) within the working range of the assay. Background-subtracted data values (mean +/- SD, n = 2) are graphed.

17. ASSAY SPECIFICITY

This kit recognizes both native and recombinant human beta Nerve Growth Factor protein in human serum, plasma, urine and cell culture supernatants.

Cell and tissue extract samples have not been tested with this kit.

CROSS REACTIVTY

The following protein were prepared at 50 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

CNTF

INTERFERENCE

The following protein were prepared at 50 ng/mL and assayed for assay interference in the presence of 875 pg/mL beta NGF. No interference was observed.

CNTF

18. SPECIES REACTIVITY

This kit recognizes human beta NGF protein.

No signal was observed in 25% serum from the following species:

- Mouse
- Rat
- Hamster
- Rabbit
- Dog
- Goat
- Pig
- Cow

Please contact our Technical Support team for more information.

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

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)