

ab326386 – Human NT-proBNP SimpleStep ELISA® Kit, Chemiluminescent

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

For overview, typical data and additional information please visit: www.abcam.com/ab326386

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, sensitivity, cell culture media validation, and healthy serum donors values were collected using the colorimetric version of this kit (ab263877).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human NT-proBNP Capture Antibody 10X	600 µL	+4°C
Human NT-proBNP Detector Antibody 10X	600 µL	+4°C
Human NT-proBNP Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Sample Diluent 50BS	20 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.

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.

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).

1. Reconstitute the NT-proBNP standard sample 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 15,000 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Add 195 µL of Sample Diluent 50BS into tube number 1 and 150 µL of Sample Diluent 50BS into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 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	195	15,000	2,000
2	Standard#1	75	150	2,000	667
3	Standard#2	75	150	667	222
4	Standard#3	75	150	222	74.1
5	Standard#4	75	150	74.1	24.7
6	Standard#5	75	150	24.7	8.2
7	Standard#6	75	150	8.2	2.7
8	Standard#7	75	150	2.7	0.92
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	≤50%
Plasma – Citrate	≤50%
Plasma – EDTA	≤50%
Plasma – Heparin	≤50%
Congestive Heart Failure Serum	0.2 – 7.5%
Cell Culture Media*	≤100%

*Media is RPMI 1640 containing 10% fetal bovine serum.

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 1:2 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 at least 1:2 into Sample Diluent 50BS 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. Assay, or dilute samples into Sample Diluent 50BS 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 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

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human NT-proBNP.

The standard protein in this kit is full-length human NT-proBNP, amino acids 27 – 102 of Uniprot ID P16860.

The antibodies in this assay do not react with the BNP-32 peptide sequence.

Native signal was detected in congestive heart failure serum and eight out of nine individual healthy donor serum samples.

Spiked protein experiments were used to validate serum, plasma, and cell culture supernatant sample types.

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

SPECIES REACTIVITY

No signal was observed in 50% normal serum samples from the following species: Mouse, Rat, Cow.

Other species reactivity not determined.

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	309	351	330
0.92	642	597	620
2.7	1,048	981	1,015
8.2	3,396	3,327	3,362
24.7	11,327	11,148	11,238
74.1	49,607	52,342	50,975
222	176,690	185,460	181,075
667	713,150	793,260	753,205
2,000	3,131,000	3,115,700	3,123,350

Table 1. Example of human NT-proBNP standard curve in Sample Diluent 50BS. The NT-proBNP 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 0.82 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of NT-proBNP 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 (%)
50% Serum	90	84 - 94
50% Plasma – Citrate	90	85 - 94
50% Plasma – EDTA	89	88 - 90
50% Plasma – Heparin	89	84 - 93
100% Cell Culture Media*	104	99 - 109
1% Congestive Heart Failure Serum	110	105 - 118

*Media is RPMI 1640 containing 10% fetal bovine serum.

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 NT-proBNP was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 50BS.

Dilution Factor	Interpolated value	7.5% Congestive Heart Failure Serum
Undiluted	pg/mL	1,218
	% Expected value	100
2	pg/mL	662
	% Expected value	109
4	pg/mL	295
	% Expected value	97
8	pg/mL	146
	% Expected value	96
16	pg/mL	82
	% Expected value	108

Recombinant NT-proBNP was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 50BS.

Dilution Factor	Interpolated value	50% Serum	50% Plasma (EDTA)	50% Plasma (Citrate)	50% Plasma (Heparin)	100% Cell Culture Media*
Undiluted	pg/mL	719	657	725	761	927
	% Expected value	100	100	100	100	100
2	pg/mL	318	288	327	354	3
	% Expected value	89	88	90	93	97
4	pg/mL	149	147	161	172	238
	% Expected value	83	90	77	90	103
8	pg/mL	74	71	85	85	116
	% Expected value	83	86	37	89	100
16	pg/mL	37	37	22	42	64
	% Expected value	83	91	99	89	111

*Media is RPMI 1640 containing 10% fetal bovine serum.

Precision

Mean coefficient of variations of interpolated values of NT-proBNP from three concentrations of spiked normal human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	4.3	5.6

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