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Human proBNP ELISA Kit

For the quantitative measurement of human pro-brain natriuretic peptide (proBNP) in serum, plasma, and cell culture supernatants.

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

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1. Overview

Abcam's Human proBNP ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Human proBNP in serum, plasma and cell culture supernatants.

This assay employs an antibody specific for Human proBNP coated on a 96-well plate. Standards and samples are pipetted into the wells and the immobilized antibody captures proBNP present in the samples. The wells are washed and biotinylated anti-Human proBNP antibody is added. After washing away any unbound biotinylated antibody, an HRP-conjugated streptavidin is pipetted to the wells. After incubation, the wells are again washed, followed by the addition of a TMB substrate solution to the wells. Color will develop in proportion to the amount of proBNP bound in each well. Addition of the Stop Solution will change the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standard or sample to each well used. Incubate at room temperature

Add prepared biotinylated antibody to each well. Incubate at room temperature.

Add prepared streptavidin solution. Incubate at room temperature.

Add TMB One-Step Development Solution to each well. Incubate at room temperature.

Add Stop Solution to each well. Read immediately.

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. 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.

6. Materials Supplied

Item	Amount	Storage Condition (Before Preparation)
Pre-coated proBNP microplate (12 x 8 well strips)	96 wells	-20°C
20X Wash Buffer Concentrate	25 mL	-20°C
Human proBNP Standard	2 vials	-20°C
5X Assay Diluent	15 mL	-20°C
Detection Antibody proBNP (biotinylated anti-Human proBNP)	2 vials	-20°C
400X HRP-Streptavidin concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution: 0.2M sulfuric acid	8 mL	-20°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

8. Technical Hints

- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- 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.
- Completely aspirate all solutions and buffers during wash steps. When preparing your standards, it is critical to briefly centrifuge the vial first. The powder may adhere to the cap and not be included in the standard solution resulting in an incorrect concentration. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and centrifuge briefly; repeat this procedure 3-4 times. This is an effective technique for thorough mixing of the standard without using excessive mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- **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 Scientific 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.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Assay Diluent

Dilute 5X Assay Diluent 5-fold with deionized or distilled water before use.

9.2 1X Wash Buffer

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into 380 mL deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.3 Detection Antibody proBNP (biotinylated anti-Human proBNP)

Briefly centrifuge the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent and used in Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly centrifuge the 400X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. The 400X HRP Streptavidin concentrate should be diluted 400-fold with 1X Assay Diluent.

For example: Briefly centrifuge the vial and pipette up and down to mix gently. Add 30 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent to prepare a 1X HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix well.

10. Standard Preparation

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Standard (recombinant protein) should be stored at -20°C or 80°C (recommended at -80°C) after reconstitution.

10.1 Briefly centrifuge a vial of Human proBNP Standards and then add 800 µL 1X Assay Diluent into the Human proBNP Standard vial to prepare a 100 ng/mL **Standard#1**. Mix thoroughly but gently.

10.2 Label tubes #2-8 and add 400 µL 1X Assay Diluent into each tube.

10.3 Prepare the 33.33 ng/mL **Standard #2** by adding 200 µL Standard #1 into tube #2. Mix thoroughly but gently.

10.4 Prepare **Standard #3** by adding 200 µL **Standard #2** to tube #3. Mix thoroughly but gently.

10.5 Using the table below as a guide, prepare further serial dilutions.

10.6 **Standard #8** contains no protein and is the Blank control.

Standard Dilution Preparation Table

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See Step 10.1				100
2	Standard #1	200	400	100	33.33
3	Standard #2	200	400	33.33	11.11
4	Standard #3	200	400	11.11	3.70
5	Standard #4	200	400	3.70	1.235
6	Standard #5	200	400	1.235	0.412
7	Standard #6	200	400	0.412	0.137
8 (Blank)	none	-	400	0	0

11. Sample Preparation

- If your samples need to be diluted, 1X Assay Diluent should be used for dilution of sera, plasma, and culture supernatants.
- Suggested dilution for sera or plasma: 2-fold. Basal expression of proBNP in normal sera is low and may not be detected in this assay.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

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 well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. 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.

13.1 Add 100 µL of each standard (see Standard Preparations, section) and sample into appropriate wells. Cover plate and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

13.2 Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with 300 µL 1X Wash Buffer using a multi-channel pipette or automatic plate washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it by tapping gently against clean paper towels.

13.3 Add 100 µL of the prepared biotinylated Human proBNP Detection Antibody (see Reagent Preparation section) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of prepared 1X HRP-Streptavidin solution (see Reagent Preparation section) to each well. Incubate for 45 minutes at room temperature with gentle shaking

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

13.8 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average Blank absorbance value. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. Typical Data

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

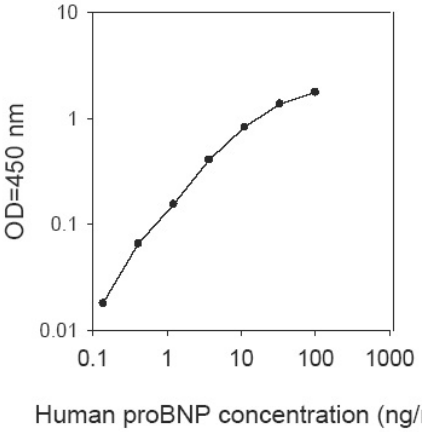

Figure 1. Example of typical Human proBNP standard curve using 1X Assay Diluent. The standard curve was prepared as described in Section 10.

16. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of proBNP is 0.14 ng/mL.

PRECISION –

	Intra-assay Precision	Inter-Assay Precision
CV (%)	<10	<12

RECOVERY –

Recovery was determined by spiking various levels of Human proBNP into Human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	139.7	126-147
Plasma	126.3	103-150
Cell culture media	129.9	110-150

Linearity of Dilution

Serum Dilution	Average % Expected Value	Range (%)
1:2	110.0	102-118
1:4	109.0	101-117

Plasma Dilution	Average % Expected Value	Range (%)
1:2	102.2	90-109
1:4	115.6	105-129

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	119.2	110-127
1:4	117.3	109-125

17. Assay Specificity

The antibodies used within this ELISA kit detect human proBNP and NT-proBNP.

Cross reaction of these antibodies with other species of proBNP has not been tested.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipette performance
	Improper standards 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 time; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
High %CV	Inaccurate pipetting	Check pipette performance
High background	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, ensure it is working properly.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.
	Stop solution	Stop solution should be added to each well before measuring

19. Notes

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