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ab136936 –
Bradykinin ELISA Kit

Instructions for Use

For quantitative detection of Bradykinin in plasma, serum and urine.

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

Version 5 Last Updated 15 January 2019

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1. BACKGROUND

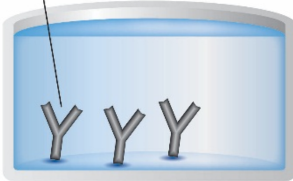
Abcam's Bradykinin *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Bradykinin in plasma, serum and urine.

A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with a solution of Bradykinin conjugated to biotin, followed by a solution of polyclonal antibody to Bradykinin. The plate is washed to remove unbound reagents. A solution of streptavidin-HRP conjugate is then added. After further incubation, the excess reagents are washed away and TMB substrate is added, which is catalyzed by HRP to generate a blue color. A stop solution changes this color from blue to yellow, and the intensity of this yellow coloration is inversely proportional to the amount of Bradykinin captured in the plate.

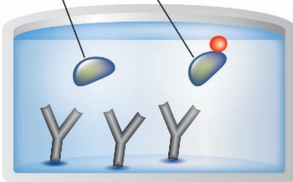
Bradykinin was discovered in 1949 as a substance generated from a globulin precursor in plasma by the action of proteases. Its name indicates that it causes a slow movement of the gut. As early as 1909 it was noted that substances found in urine, which were later identified as kinins, have hypotensive actions.

Kinins are effectors of vasodilation, vascular permeability, NO release and arachidonic acid mobilization. They are important regulators of blood pressure, kidney function and heart function, and they are also involved in inflammation. Bradykinin is generated from the blood globulin Kininogen HK, by the action of the kallikrein system in blood (related to the blood clotting cascade) but can also be generated in other tissues and organs. Besides kallikrein, other proteases such as plasmin may also release bradykinin. Several peptidases can degrade kinins, including Angiotensin Converting Enzyme (ACE), a metalloproteinase which converts Angiotensin I to Angiotensin II and destroys bradykinin. Plasma Bradykinin is rapidly degraded to a smaller stable peptide (BK1-5) form.

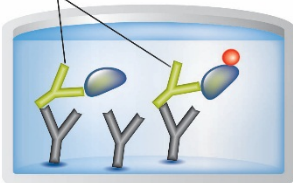
2. ASSAY SUMMARY



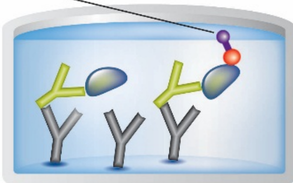
Prepare all reagents and samples as instructed.



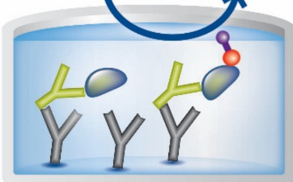
Add samples and biotinylated antigen and to appropriate wells. Incubate at room temperature.



Add bradykinin polyclonal antibody to appropriate wells. Incubate at room temperature.



Wash and add prepared labeled streptavidin HRP-conjugate to appropriate wells. Incubate at room temperature.



Wash and add TMB substrate 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.

- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up
- Stop Solution is a solution of HCl. This solution is caustic; care should be taken in use
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results

4. STORAGE AND STABILITY

Store kit at +4°C immediately upon receipt, apart from the Bradykinin Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Goat anti-Rabbit IgG Microplate (12 x 8 wells)	96 wells	+4ºC
Bradykinin Conjugate	5 mL	+4ºC
Bradykinin Antibody	5 mL	+4ºC
Bradykinin Standard	2 Vials	-20ºC
Assay Buffer 16	55 mL	+4ºC
20X Wash Buffer Concentrate	27 mL	+4ºC
TMB Substrate	2 x 10 mL	+4ºC
Stop Solution 2	10 mL	+4ºC
Streptavidin-HRP	12.5 µg	+4ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Standard microplate reader - capable of reading at 450 nm,
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- Deionized or distilled water

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

- Standards can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the well to avoid contamination.
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
- Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.
- **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 and samples to room temperature (18 - 25°C) prior to use.

9.1 1X Streptavidin-HRP

Reconstitute one vial of Streptavidin-HRP with 250 µL of deionized water and vortex thoroughly. Store at 4ºC for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles. Prepare the working concentration by diluting stock 1:1000 in the assay buffer. Do not store diluted Streptavidin-HRP.

9.2 1X Wash Buffer

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 30 minutes of preparation.

10.1 Reconstitute 1 vial of Bradykinin standard with 1 mL of Assay Buffer. This is **standard 1**. Vortex to ensure the entire cake is dissolved.

10.2 Label 5 tubes #2 – #6.

10.3 Add 900 µL Assay Buffer into tube #2.

10.4 Add 750 µL assay buffer into tubes #3 to #6.

10.5 Prepare **Standard 2** by transferring 100 µL from Standard 1 to tube #2. Mix thoroughly and gently.

10.6 Prepare **Standard 3** by transferring 250 µL from tube #2 to tube #3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes #4 through to tube #6.

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Standard	See Step 10.1			30,000
2	Standard 1	100	900	30,000	3,000
3	Standard 2	250	750	3,000	750
4	Standard 3	250	750	750	187.5
5	Standard 4	250	750	187.5	46.9
6	Standard 5	250	750	46.9	11.7

11. SAMPLE COLLECTION AND STORAGE

- The assay is suitable for the measurement of Bradykinin serum, plasma and urine. This kit is not species specific. However, samples containing rabbit IgG will interfere in the assay due to the goat anti- rabbit IgG coated plate. Prior to assay, frozen samples should be brought to 4°C and centrifuged, if necessary, to isolate residual debris.
- A minimum 1:16 dilution is required for plasma, urine and serum samples. This is the minimum recommended dilution necessary to remove matrix interference in the assay. Due to differences in individual samples, users must determine the optimal sample dilution for their particular experiments

  Note: The short half-life of Bradykinin may lead to variability in serum results.

11.1 Protocol for Plasma samples

11.1.1 Collect whole blood in an ice cold tube containing Sodium EDTA.

11.1.2 Mix blood in a ratio of 1:4 with ice cold ethanol.

11.1.3 Centrifuge at 1000 x g for 15 minutes at 4°C.

11.1.4 Remove ethanol prepared plasma to a clean plastic tube.

11.1.5 Sample should be divided into aliquots and frozen within 2 hours of collection at or below -20°C. Samples may be stored frozen for up to 2 weeks or proceed with the sample preparation.

11.1.6 Dry down sample and reconstitute with the provided assay buffer prior to use in this assay.

11.2 Protocol for Serum Samples

11.2.1 Collect whole blood in appropriate serum tubes

11.2.2 Incubate upright at room temperature for 30–45 minutes to allow clotting to occur.

11.2.3 Centrifuge at 1000 x g for 15 minutes at 4°C. Do not use brake.

11.2.4 Without disturbing the cell layer, place supernatant into clean tube containing protease inhibitor cocktail to a final concentration of 0.05% and PMSF to a final concentration of 1mM.

11.2.5 The supernatant may be divided into aliquots and stored at or below -20°C, or used immediately in the assay.

11.2.6 Samples may be stored for up to two weeks.

11.2.7 Avoid repeated freeze-thaw cycles

11.3 Protocol for Urine Samples

11.3.1 Collect spontaneous or 24 hour urine in a bottle containing 10-15mL of 6N HCl as a preservative.

11.3.2 Urine should be mixed in a ratio of 1:4 with glacial acetic acid.

11.3.3 Centrifuge at 1500 x g for 30 minutes at 4°C.

11.3.4 Remove lower phase and transfer to a clean plastic tube.

11.3.5 Using the material collected in step 4, repeat steps 3 and 4 twice, adjusting centrifuge time to 15 minutes.

11.3.6 Sample may be divided into aliquots and stored at or below -20°C, or proceed with the sample preparation.

11.3.7 Dry down sample and reconstitute with the included assay buffer prior to use in this 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 well strips should be returned to the plate packet and stored at 4°C
- 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

Recommended plate layout:

	1	**2**	**3**	**4**
A	B_s	Std 2	Std 6	Sample 4
B	B_s	Std 2	Std 6	Sample 4
C	NSB	Std 3	Sample 1	etc
D	NSB	Std 3	Sample 1	etc
E	B_0	Std 4	Sample 2	
F	B_0	Std 4	Sample 2	
G	Std 1	Std 5	Sample 3	
H	Std 1	Std 5	Sample 3	

Plate layout shows controls, blanks and standards required for each assay. Use additional strips of wells to assay all your samples.

Key:

B_s = Blank; contains substrate only.

NSB = Non-specific binding; contains assay buffer, conjugate, SA-HRP and substrate.

B_0 = 0 pg/mL standard; contains assay buffer, conjugate, antibody, SA-HRP and substrate.

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**
- **Refer to the recommended plate layout in Section 12 before proceeding with the assay**

13.1 Add 150 µL of the Assay Buffer into the NSB (nonspecific binding) wells.

13.2 Add 100 µL of Assay Buffer into the B_0 (0ng/mL standard) wells.

13.3 Add 100 µL of prepared standards #1 to #6 and 100 µL diluted samples to the appropriate wells.

13.4 Add 50 µL of Bradykinin Conjugate to NSB, B_0, standard and sample wells i.e. not B_s (blank) wells.

13.5 Add 50 µL of Bradykinin Antibody to B_0, standard and sample wells i.e. not B_s and NSB wells.

13.6 Seal the plate. Incubate the plate at room temperature for 2 hours on a plate shaker (~500 rpm*).

13.7 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.8 Add 200 µL of 1X Streptavidin-HRP to NSB, B_0, standard and sample wells i.e. not B_s wells.

13.9 Seal the plate. Incubate the plate at room temperature for 30 minutes on a plate shaker (~500 rpm).

13.10 Wash as described in step 13.7.

13.11 Add 200 µL of the TMB Substrate solution to all wells. Incubate at room temperature for 30 minutes without shaking.

13.12 Add 50 µL Stop Solution into each well. The plate should be read immediately.

13.13 After blanking the plate reader against the B_s (blank) wells, read the O.D. absorbance at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

*The optimal speed for each shaker will vary. The speed must be set to ensure adequate mixing of the wells, but not so vigorously that the contents of the wells splash out and contaminate other wells.

14. CALCULATIONS

14.1 Calculate the average net absorbance measurement (Average Net OD) for each standard and sample by subtracting the average NSB absorbance measurement from the average absorbance measurement (Average OD) for each standard and sample.

Average Net OD = Average OD - Average NSB OD

14.2 Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (B_0), using the following formula:

$$\text{Percent Bound} = \frac{\text{Average Net OD}}{\text{Average Net } B_0 \text{ OD}} \times 100$$

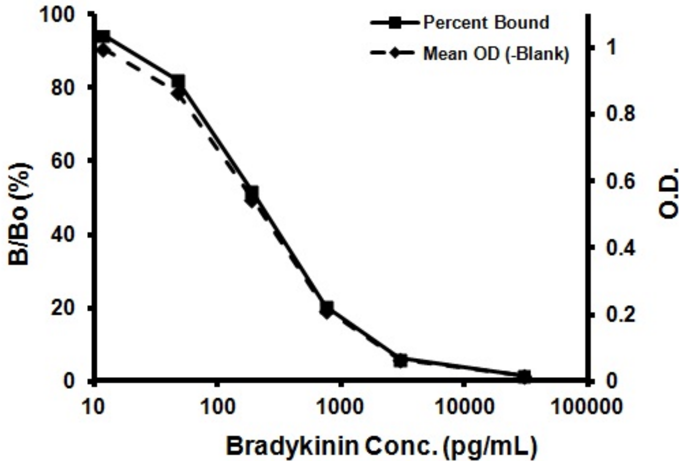
14.3 Plot the Percent Bound (B/B_0) versus concentration of bradykinin for the standards. Fit a line through the data. The concentration bradykinin in the unknowns can be determined by interpolation.

Note: A four parameter algorithm (4PL) provides the best fit, though other algorithms can be examined to see which provides the most accurate fit of the data (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.



Sample	Mean OD (-Bs)	% Bound	Bradykinin pg/mL
B_s	(0.04)	-	-
NSB	0.004	0	-
Standard 1	0.016	1.5	30,000
Standard 2	0.065	6.2	3,000
Standard 3	0.215	20.3	750
Standard 4	0.548	51.9	187.5
Standard 5	0.869	82.3	46.9
Standard 6	0.996	94.2	11.7
Bo	1.057	100	0.0
Unknown 1	0.244	23.0	637.6
Unknown 2	0.78	73.8	75.5

Typical Quality Control Parameters –

Quality of Fit = 1.0000 (Calculated from 4 parameter logistic curve fit)

20% Intercept = 759 pg/mL

50% Intercept = 202 pg/mL

80% Intercept = 54 pg/mL

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity, minimum detectable dose of Bradykinin using this ELISA kit was found to be 24.8 pg/mL. This was determined by the average optical density of the 0 pg/mL Standard and comparing to the average optical density for Standard 6. The detection limit was determined as the concentration of Bradykinin measured at two standard deviations from the zero along the standard curve.

LINEARITY OF DILUTION –

Human samples containing Bradykinin were serially diluted 1:4 in the kit assay buffer and measured in the assay. The results are shown in the table below.

	% of Expected		
Dilution	**Human Plasma**	**Human Serum**	**Human Urine**
1:4	93	111	-
1:16	107	108	100
1:64	-	100	-
1:256	-	-	-

SAMPLE RECOVERY –

Individually diluted samples were prepared to read within the dynamic range of the assay. Next recombinant Bradykinin was spiked into these samples at three different concentrations. Endogenous Bradykinin was subtracted from the spiked values and the average recovery in each of the spiked matrices was compared to the recovery of identical spikes in the assay buffer. The mean and the range percent recovery at the three different concentrations are indicated below for each matrix.

Sample	Dilution	Spike Concentration (pg/mL)	% Recovery of Spike	Range of recovery %
Human Plasma	1:16	20,000	130	113-155
		2,000	102	101-104
		100	98	14-148
Human Urine	1:16	2,000	99	97-102
		100	88	81-100
		10	139	58-279
Human Serum	1:64	2,000	103	94-112
		100	123	48-184

PRECISION –

Intra-assay

Intra-assay precision was determined by assaying 20 replicates of 3 buffer controls containing Bradykinin in a single assay.

	Bradykinin (pg/mL)	%CV
Low	73.7	9.9
Medium	208.7	6.2
High	695.4	4.6

Inter-assay

Inter-assay precision was determined by measuring buffer controls of varying Bradykinin concentrations in multiple assays over several days.

	Bradykinin (pg/mL)	%CV
Low	66.1	11.9
Medium	209.3	10.3
High	700.4	15.0

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

The cross reactivities for a number of related compounds were determined by diluting the cross reactants to concentrations in the range of 0.1 pM to 500 nM. These samples were then measured in the assay.

Analyte	% Cross Reactivity
Bradykinin	100
Lys-Bradykinin (Kallidin)	100
Les-Des-Arg9-Bradykinin	<1
BK1-5 stable degradation product	<0.1

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	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 times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
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 kit	Store the all components as directed

19. NOTES

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