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.
INTRODUCTION

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 yellow color. A stop solution changes this color from yellow to blue, and the intensity of this blue 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**

**Capture Antibody**

Prepare all reagents and samples as instructed.

**Sample**

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

**Biotin Labeled Conjugate**

**Target Antibody**

Add Bradykinin Polyclonal Antibody to appropriate wells. Incubate at room temperature.

**Streptavidin-HRP**

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

**Substrate**

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat anti-Rabbit IgG Microplate (12 x 8 wells)</td>
<td>96 Wells</td>
<td>4°C</td>
</tr>
<tr>
<td>Bradykinin Conjugate</td>
<td>5 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Bradykinin Antibody</td>
<td>5 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Bradykinin Standard</td>
<td>2 Vials</td>
<td>-20°C</td>
</tr>
<tr>
<td>Assay Buffer 16</td>
<td>55 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>20X Wash Buffer Concentrate</td>
<td>27 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>2 x 10 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Stop Solution 2</td>
<td>10 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Streptavidin-HRP</td>
<td>12.5 µg</td>
<td>4°C</td>
</tr>
</tbody>
</table>
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 405 nm, preferably with correction between 570 and 590 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 must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. 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 4°C 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 the assay buffer. This is **standard 1**. Vortex to ensure the entire cake is dissolved.

10.2 Label five tubes with numbers 2 – 6 and another tube $B_O$.

10.3 Add 750 μL assay buffer into all tubes.

10.4 Prepare **Standard 2** by transferring 900 μL from Standard 1 to tube 2 to tube 2. Mix thoroughly and gently.

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

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

10.7 **Standard $B_O$** contains no protein and is the Blank Activity control.
## ASSAY PREPARATION

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Sample to Dilute</th>
<th>Volume to Dilute (µL)</th>
<th>Volume of Diluent (µL)</th>
<th>Starting Conc. (pg/mL)</th>
<th>Final Conc. (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard</td>
<td>See Step 10.1</td>
<td></td>
<td>30,000</td>
<td>30,000</td>
</tr>
<tr>
<td>2</td>
<td>Standard 1</td>
<td>100</td>
<td>900</td>
<td>30,000</td>
<td>3,000</td>
</tr>
<tr>
<td>3</td>
<td>Standard 2</td>
<td>250</td>
<td>750</td>
<td>3,000</td>
<td>750</td>
</tr>
<tr>
<td>4</td>
<td>Standard 3</td>
<td>250</td>
<td>750</td>
<td>750</td>
<td>187.5</td>
</tr>
<tr>
<td>5</td>
<td>Standard 4</td>
<td>250</td>
<td>750</td>
<td>187.5</td>
<td>46.9</td>
</tr>
<tr>
<td>6</td>
<td>Standard 5</td>
<td>250</td>
<td>750</td>
<td>46.9</td>
<td>11.7</td>
</tr>
<tr>
<td>B₀</td>
<td>None</td>
<td>-</td>
<td>750</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

![Tube diagram](image-url)
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 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
- It is recommended to assay all standards, controls and samples in duplicate
- Two sets of replicate wells must be used to measure $B_0$ (Blank Activity), Total Activity (TA) and Non Specific Binding (NSB)

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

13.2 Add 100 µL of all standards, $B_0$, and samples to the appropriate wells.

13.3 Add 50 µL of Bradykinin Conjugate to all wells.

13.4 Add 50 µL of Bradykinin antibody to all wells.

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

13.6 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.

13.7 Add 200 µL of 1X Streptavidin-HRP to each well except the blank.

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

13.9 Wash as described in step 13.6.

13.10 Add 200 µL of the TMB Substrate solution to every well. Incubate at room temperature for 30 minutes without shaking.

13.11 Add 50 µL Stop Solution into each well. The plate should be read immediately.
13.12 Read the O.D. absorbance at 450 nm, preferably with correction between 570 and 590 nm.
14. Calculations

A four parameter algorithm (4PL) 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). Values should be expressed as a percentage of the B\textsubscript{0}. Divide the replicate average sample or standard OD measurement by the replicate average B\textsubscript{0} measurement. 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.

- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

\[
\text{Average Net OD} = \text{Average Bound OD} - \text{Average NSB OD}
\]

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

\[
\text{Percent Bound} = \left(\frac{\text{Net OD}}{\text{Net Bo OD}}\right) \times 100
\]

- Plot both the Percent Bound and the Net OD versus Concentration of Bradykinin for the standards. Sample concentrations may be calculated off of Net OD values using the desired curve fitting
15. TYPICAL DATA

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean OD (-Blank)</th>
<th>% Bound</th>
<th>Bradykinin pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1</td>
<td>0.016</td>
<td>1.5</td>
<td>30,000</td>
</tr>
<tr>
<td>Standard 2</td>
<td>0.065</td>
<td>6.2</td>
<td>3,000</td>
</tr>
<tr>
<td>Standard 3</td>
<td>0.215</td>
<td>20.3</td>
<td>750</td>
</tr>
<tr>
<td>Standard 4</td>
<td>0.548</td>
<td>51.9</td>
<td>187.5</td>
</tr>
<tr>
<td>Standard 5</td>
<td>0.869</td>
<td>82.3</td>
<td>46.9</td>
</tr>
<tr>
<td>Standard 6</td>
<td>0.996</td>
<td>94.2</td>
<td>11.7</td>
</tr>
<tr>
<td>Bo</td>
<td>1.057</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>Unknown 1</td>
<td>0.244</td>
<td>23.0</td>
<td>637.6</td>
</tr>
<tr>
<td>Unknown 2</td>
<td>0.78</td>
<td>73.8</td>
<td>75.5</td>
</tr>
</tbody>
</table>
DATA ANALYSIS

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 Abcam 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.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>% of Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human Plasma</td>
</tr>
<tr>
<td>1:4</td>
<td>93</td>
</tr>
<tr>
<td>1:16</td>
<td>107</td>
</tr>
<tr>
<td>1:64</td>
<td>-</td>
</tr>
<tr>
<td>1:256</td>
<td>-</td>
</tr>
</tbody>
</table>
DATA ANALYSIS

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.

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

PRECISION –

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

<table>
<thead>
<tr>
<th>Bradykinin (pg/mL)</th>
<th>Intra-Assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>73.7</td>
</tr>
<tr>
<td>Medium</td>
<td>208.7</td>
</tr>
<tr>
<td>High</td>
<td>695.4</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Bradykinin (pg/mL)</th>
<th>Inter-Assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>66.1</td>
</tr>
<tr>
<td>Medium</td>
<td>209.3</td>
</tr>
<tr>
<td>High</td>
<td>700.4</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Cross Reactivities in the range of 0.1pM – 500nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td>100</td>
</tr>
<tr>
<td>Lys-Bradykinin (Kallidin)</td>
<td>100</td>
</tr>
<tr>
<td>Les-Des-Arg9-Bradykinin</td>
<td>&lt;1</td>
</tr>
<tr>
<td>BK1-5 stable degradation product</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>
## 18. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standards dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; change to overnight standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td>Samples give higher value than the highest standard</td>
<td>Starting sample concentration is too high.</td>
<td>Dilute the specimens and repeat the assay</td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the kit</td>
<td>Store the all components as directed</td>
</tr>
</tbody>
</table>
19. **NOTES**
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