Bradykinin ELISA kit

For quantitative determination of bradykinin in serum, plasma or urine samples.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	74-BRAHU-E01
Size:	96 Wells
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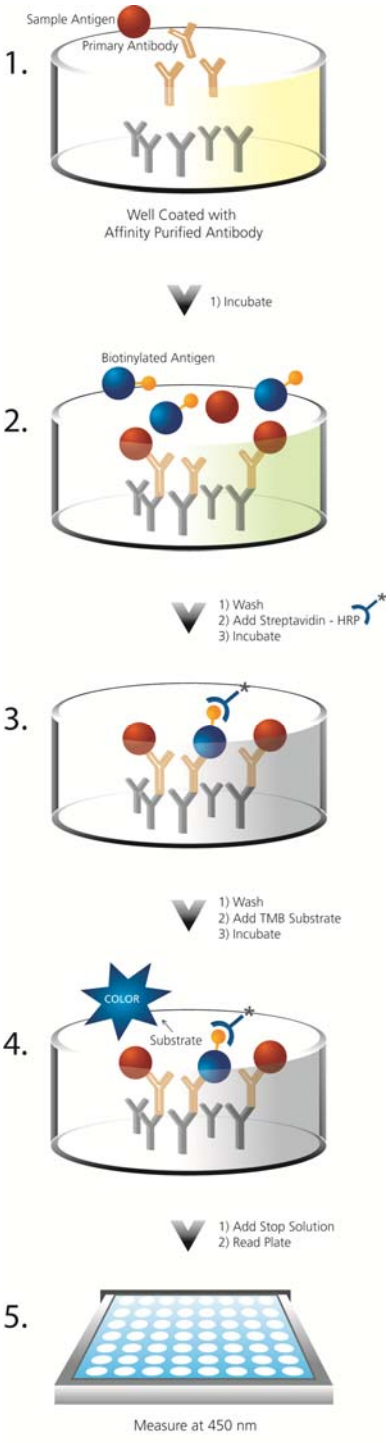


Introduction

The Bradykinin Enzyme-Linked Immunosorbent Assay (ELISA) kit is a complete kit for the quantitative determination of Bradykinin in plasma, serum and urine. Please read the entire kit insert before performing this assay.

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[1]. As early as 1909 it was noted that substances found in urine, which were later identified as kinins, have hypotensive actions[2].

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[2,3,5].

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[3]. 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[3,5]. Plasma Bradykinin is rapidly degraded to a smaller stable peptide (BK1-5) form[4].

Principle

1. Standards and samples are added to wells coated with a GxR IgG antibody. A blue solution of Bradykinin conjugated to biotin is then added to the wells.
2. A yellow solution of rabbit polyclonal antibody to Bradykinin is then added, and the plate is incubated at room temperature. During this incubation the antibody binds, in a competitive manner, the Bradykinin in the sample or conjugate. The plate is then washed, leaving only bound Bradykinin.
3. A solution of streptavidin conjugated to horseradish peroxidase is added to each well, to bind the biotinylated Bradykinin. The plate is again incubated.
4. The plate is washed to remove excess HRP conjugate. TMB substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is inversely proportional to the level of Bradykinin in the sample.

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known and unknown effects of the antigen.

Materials Supplied

1. **Assay Buffer 16**
   55 mL
   Tris buffer containing proteins and preservative

2. **Bradykinin Standard**

   Two vials containing 30,000pg lyophilized Bradykinin

3. **Goat anti-Rabbit IgG Microtiter Plate**
   One plate of 96 wells
   A clear plate of break-apart strips coated with a goat anti-rabbit polyclonal antibody

4. **Bradykinin Antibody**
   5 mL
   A yellow solution of polyclonal antibody to Bradykinin

5. **Bradykinin Conjugate**
   5 mL
   A blue solution of biotinylated Bradykinin

6. **Streptavidin-HRP**

   One vial containing 12.5 µg of lyophilized streptavidin conjugated to horseradish peroxidase.

7. **Wash Buffer Concentrate**
   27 mL
   Tris buffered saline containing detergents

8. **TMB Substrate**

   Two bottles containing 10 mL each
   A solution of 3,3'5,5' tetramethylbenzidine (TMB) and hydrogen peroxide

9. **Stop Solution 2**
   10 mL
   A 1N solution of hydrochloric acid in water

10. **Bradykinin Assay Layout Sheet**
    1 each

11. **Plate Sealer**
    2 each

Protect substrate from prolonged exposure to light.

Stop solution is caustic. Keep tightly capped.

Reagents require separate storage conditions.

Storage

All components of this kit, **except the Standard**, should be stored at 4°C upon receipt. The Standard should be stored at **-20°C**. Shipping conditions may not reflect storage conditions.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1000 µL.
3. Repeater pipet for dispensing 50 µL and 200 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. Microplate shaker.
7. Lint-free paper toweling for blotting.
8. Microplate reader capable of reading at 450 nm.
9. Graph paper for plotting the standard curve.

Sample Handling

The assay is suitable for the measurement of Bradykinin in plasma, serum and urine. Due to the conserved nature of Bradykinin between species, 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 use, 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.

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Protocol for Plasma

1. Collect whole blood in an ice cold tube containing Sodium EDTA.
2. Mix blood in a ratio of 1:4 with ice cold ethanol.
3. Centrifuge at 1000 x g for 15 minutes at 4°C.
4. Remove ethanol prepared plasma to a clean plastic tube.
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.
6. Dry down sample and reconstitute with the provided assay buffer prior to use in this assay.

Protocol for Urine

1. Collect spontaneous or 24 hour urine in a bottle containing 10 – 15mL of 6N HCl as a preservative.
2. Urine should be mixed in a ratio of 1:4 with glacial acetic acid.
3. Centrifuge at 1500 x g for 30 minutes at 4°C.
4. Remove lower phase and transfer to a clean plastic tube.
5. Using the material collected in step 4, repeat steps 3 and 4 twice, adjusting centrifuge time to 15 minutes.
6. Sample may be divided into aliquots and stored at or below -20°C, or proceed with the sample preparation.
7. Dry down sample and reconstitute with the included assay buffer prior to use in this assay.

Protocol for Serum

1. Collect whole blood in appropriate serum tubes
2. Incubate upright at room temperature for 30-45 minutes to allow clotting to occur.
3. Centrifuge at 1000 x g for 15 minutes at 4°C. Do not use brake.
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.
5. The supernatant may be divided into aliquots and stored at or below -20°C, or used immediately in the assay.
6. Samples may be stored for up to two weeks.
7. Avoid repeated freeze-thaw cycles.

Sample Recoveries

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 Matrix	Dilution	Spike Concentration	Recovery of Spike (Range)
Human Plasma (n=5)	1:16	20000 pg/mL	130% (113-155)
		2000 pg/mL	102% (101-104)
		100 pg/mL	98% (14-148)
Human Urine (n=3)	1:16	2000 pg/mL	99% (97-102)
		100 pg/mL	88% (81-100)
		10 pg/mL	139% (58-279)
Human Serum (n=4)	1:64	2000 pg/mL	103% (94-112)
		100 pg/mL	123% (48-184)

Glass or polypropylene tubes may be used for standard preparation. Avoid polystyrene.

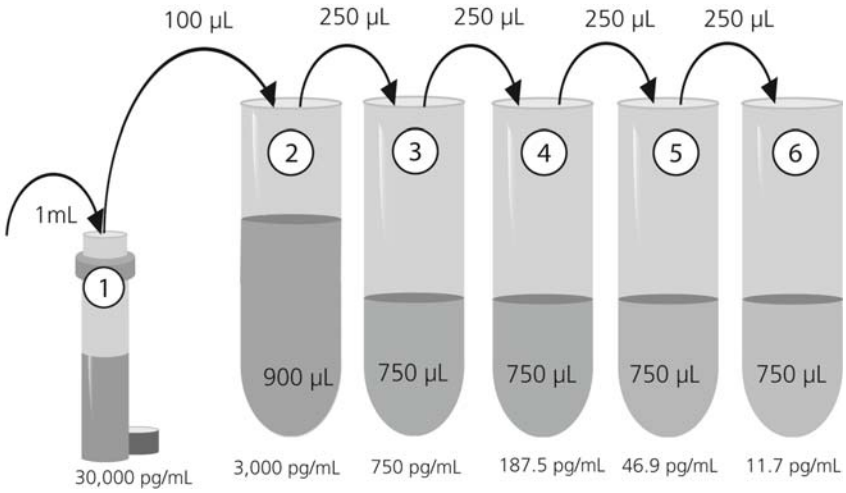
Reagent Preparation

1. **Wash Buffer**

   Prepare the wash buffer by diluting 5 mL of the supplied Wash Buffer Concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **Bradykinin Standard**

   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. Label five 12 x 75 mm tubes #2 through #6. Pipet 900 µL of the assay buffer into tube #2, pipet 750 µL of the assay buffer into tube #3. Pipet 750 µL of assay buffer into tubes #4 through #6. Remove 100 µL from the reconstituted standard vial and add to tube #2, this is standard #2. Vortex thoroughly. Add 250 µL from tube #2 to tube #3. Vortex thoroughly. Continue this for tubes #4 through #6.



   Diluted standards should be used within 30 minutes of preparation. The concentrations of Bradykinin in the tubes are labeled above.

3. **Streptavidin-HRP**

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

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

All standards and samples should be run in duplicate.

Add the reagents to the side of the well to avoid contamination.

Prior to the addition of substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 150 µL of the assay buffer into the NSB (non-specific binding) wells.
2. Pipet 100 µL of the assay buffer into the Bo (0 ng/mL standard) wells.
3. Pipet 100 µL of Standards #1 through #6 to the bottom of the appropriate wells.
4. Pipet 100 µL of the samples to the bottom of the appropriate wells.
5. Pipet 50 µL of the conjugate into each well except the Blank wells.
6. Pipet 50 µL of the antibody into each well except the Blank and NSB wells.
7. Seal the plate. Incubate for 2 hours on a plate shaker (~500 rpm*) at room temperature.
8. Empty the contents of the wells and wash by adding 400 µL of wash buffer to every well. Repeat 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.
9. Pipet 200 µL of the streptavidin-HRP conjugate to each well except the Blank.
10. Seal the plate. Incubate for 30 minutes on a plate shaker (~500 rpm*) at room temperature.
11. Wash as above (Step 8).
12. Add 200 µL of the substrate solution into each well.
13. Incubate for 30 minutes at room temperature without shaking.
14. Pipet 50 µL of the stop solution into each well.
15. After blanking the plate reader against the substrate blank, read optical density at 450 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

* The plate shaker speed was based on a BellCo Mini Orbital Shaker (mod no. 7744-08096). The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.

Calculation of Results

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of Bradykinin in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. Assay Blaster! assay analysis software is an easy-to-use and cost effective program that provides the options of point-to-point, 4PL and 5PL curve fitting options.

The concentration of Bradykinin can be calculated as follows.

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

$$\text{Average Net OD} = \text{Average OD} - \text{Average NSB OD}$$

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

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

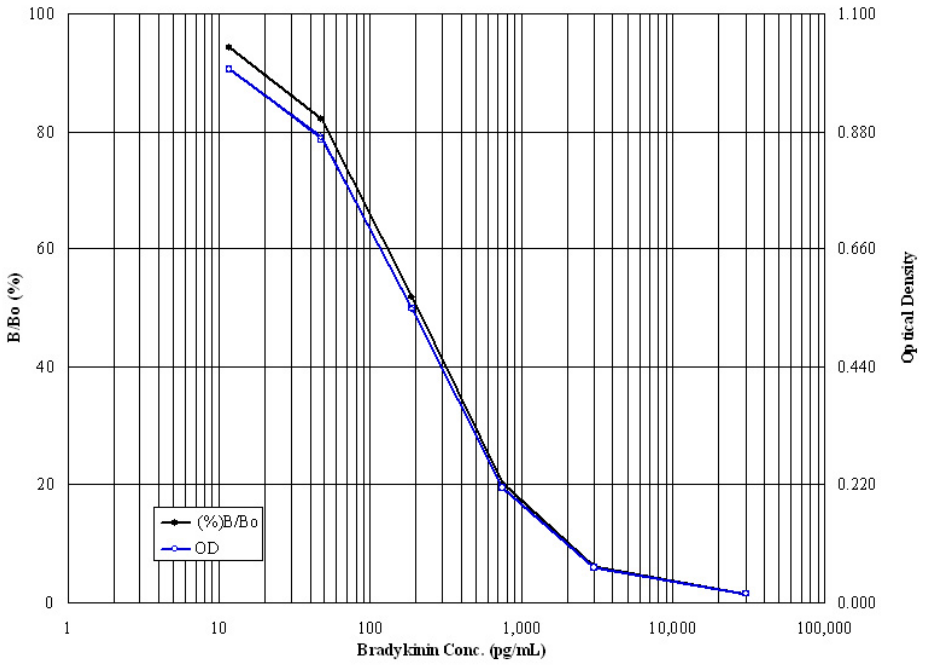
3. Plot the Percent Bound (B/Bo) versus concentration of Bradykinin for the standards. Approximate a straight line through the points. The concentration of Bradykinin of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Average Net OD	Percent Bound	Bradykinin (pg/mL)
Blank (mean)	(0.04)	---	---
NSB	0.004	0%	---
Bo	1.057	100%	0.0
S1	0.016	1.5%	30000.0
S2	0.065	6.2%	3000.0
S3	0.215	20.3%	750.0
S4	0.548	51.9%	187.5
S5	0.869	82.3%	46.9
S6	0.996	94.2%	11.7
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

Performance Characteristics

Specificity

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	Sequence	Percent cross-reactivities in the range of 0.1 pM - 500 nM
Bradykinin	RPPGFSPFR	100%
Lys-Bradykinin (Kallidin)	KRPPGFSPFR	100%
Les-Des-Arg9-Bradykinin	KRPPGFSPF	<1%
BK1-5 stable degradation product	RPPGF	<0.1%

Sensitivity

The sensitivity, defined as 2 standard deviations from the mean signal at zero, was determined from 12 independent standard curves. The standard deviation was determined from 12 zero standard replicates. The sensitivity of the assay was determined to be 24.8 pg/mL.

Parallelism and Dilutional Linearity

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.

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

Precision

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

pg/mL	%CV
695.4	4.6
208.7	6.2
73.7	9.9

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

pg/mL	%CV
700.4	15.0
209.3	10.3
66.1	11.9

References

1. Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. Rocha E Silva M, Beraldo WT, Rosenfeld G. Am J Physiol. 1949 Feb;156(2):261-73.
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Notes

Notes

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