

KAMIYA BIOMEDICAL COMPANY

Human Bradykinin EIA

**For the quantitative determination of Bradykinin
in human urine or blood samples.**

Cat. No. KT-386

For Research Use Only.

PRODUCT INFORMATION**Human Bradykinin EIA**
Cat. No. KT-386**INTENDED USE**

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INTRODUCTION

Kallidinogenase, a cardiovascularly active substance, is one of the kininogenases that releases kinins by specifically acting on kininogens. It has been widely used in medical treatment as a cardiovascular drug, such as an antihypertensive agent. It is generally acknowledged that the measurement of kinin-degrading enzymes (kininases) and kinin-releasing activity in bulk substance or preparations is important in kallidinogenase preparation manufacturing process and quality control. Kininases are determined by bioassay methods with Bradykinin (BK) as a substrate using the rat uterus or guinea pig ileum. For the measurement of kinin-releasing activity, the bioassay method is used in which the formed kinin is determined using kininogen as a substrate. However, these bioassay procedures are complicated and are labor intensive. Therefore, we developed this assay for the quantitative determination of Bradykinin based on enzyme immunoassay (EIA) to be less complicated and less time consuming.

- 1) For the determination of Bradykinin based on EIA using microstrip wells.
- 2) A convenient assay using standard ELISA equipment (microplate reader, microplate washer, etc).
- 3) This assay is highly efficient for the determination of multiple samples.
- 4) The sensitivity (lower limit of detection) is 0.173 ng/mL (7.2 pg/well).
- 5) Can determine Bradykinin concentration in samples with high specificity and accuracy.

PRINCIPLE

Sample Bradykinin and peroxidase-labeled Bradykinin are allowed to react competitively with anti-Bradykinin Antibody (rabbit) and is captured by anti-rabbit IgG antibody (goat) coated on the microstrip well. The Bradykinin concentration is determined from the enzyme activity of peroxidase-labeled Bradykinin bound to anti-Bradykinin antibody.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Bradykinin Calibrator	Lyophilized	1 vial (100 ng)	Bradykinin (for 1 mL)
2. Buffer Solution A	Liquid	1 bottle (30 mL)	
3. Buffer Solution B	Liquid	1 bottle (30 mL)	
4. Deproteinizing Reagent	Liquid	1 bottle (15 mL)	Trichloroacetic acid, 200 mg/mL
5. Bradykinin Antibody Concentrate	Lyophilized	1 vial (0.15 mL)	Rabbit anti-Bradykinin antibody, (for 15 mL)
6. Antibody-Coated Plate		1 96-well plate	Goat anti-rabbit IgG antibody
7. Wash Buffer Concentrate	Liquid	2 bottles (30 mL each)	
8. Bradykinin Enzyme Conjugate	Lyophilized	1 vial (0.08 mL)	Horseradish peroxidase-labeled Bradykinin (for 8 mL)
9. Substrate Tablet	Tablet	2 tablets (13 mg each)	o-Phenylenediamine dihydrochloride
10. Substrate Dilution Buffer	Liquid	2 bottles (15 mL each)	Hydrogen peroxide
11. Stop Solution	Liquid	1 bottle (15 mL)	

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader)
- Centrifuge (capable of > 1,000 x g)
- Washing device for microtiter plate and dispenser with an aspiration system
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips, reservoirs
- 500 mL Graduated cylinder
- Microtiter plate rotator
- Plastic test tubes for pretreatment
- Stopwatch
- Paper towels, aluminum foil, etc.
- Graph paper

PRECAUTIONS

General Precautions

1. In order to obtain reliable and consistent results, the instructions must be strictly followed.
2. Do not use the kit reagents after the expiration date.
3. Do not mix the Bradykinin Enzyme Conjugate, the Bradykinin Antibody and the Antibody-Coated Plate from kits of different lots.
4. White powder may adhere on the Antibody-Coated Plate. This will not influence the results.
5. Handle the Deproteinizing Reagent with care because its main ingredient, trichloroacetic acid, is very corrosive to skin.
6. Be sure to handle the Stop Solution with care because it contains sulfuric acid and is very corrosive.
7. If the kit reagents come in contact with skin, mouth or eyes, irrigate with water and seek medical attention immediately.
8. Carefully read the instructions of the instruments or equipments required for the assay.
9. Use the kit carefully under the supervision of specialists or leaders with sufficient knowledge of safety regarding biological research and experiments.

Procedural Precautions

1. Assaying all samples in duplicate is recommended until end-user is accustomed to the procedure.
2. Add the reagents in the order as directed in the Assay Protocol. Proceed with samples and prepared Calibrator solutions simultaneously, under the same conditions.
3. When two or more Substrate Tablets are used simultaneously, dissolve each tablet separately in separate bottles of Substrate Diluent Buffer, then mix in another container before use.
4. Do not damage or soil the bottom of the Antibody-Coated Plate. The wells are used as the cuvettes to measure the absorbance.
5. Do not foam or scatter working solutions in the wells to prevent contamination between the wells.
6. Avoid contamination between the kit reagents, or contamination of microbes in the samples and the kit reagents

Safety Precautions

1. Viral: Since no human serum or plasma is used in this kit, there is no risk from infection by HBV, HIV and HCV from the kit reagents. However, the kit reagents should be handled with the same precautions as any other potentially biohazardous materials. When determination is complete, inactivate viruses in samples, reagents and equipment by one of the following methods:
 - a. Autoclave (121°C for 20 minutes or 115°C for 30 minutes)
 - b. Submerge in sodium hypochlorite (available chlorine at 25,000 ppm for 30 minutes or 60 minutes at 10,000 ppm overnight)
 - c. Submerge in 2% glutaraldehyde for at least 1 hour
2. Do not mouth pipette reagents or samples at any time.

Waste Disposal:

Waste solutions should be treated using the same methods outlined above under "Safety Precautions / 1. Viral" and discarded with large quantities of water.

REAGENT PREPARATION

1. Calibrator Solutions:
 - a. Set up seven test tubes.
 - b. Reconstitute the Bradykinin Calibrator with exactly 1.0 mL of purified water. Mix gently to dissolve the contents completely, and transfer into a test tube. The concentration is now equivalent to 5,000 pg/well (50 μ L/well of the 100 ng/mL reconstituted calibrator).
 - c. Add 1.5 mL of Buffer Solution A to a test tube and add 0.5 mL of reconstituted Calibrator Solution at 5,000 pg/well. Mix well. This Calibrator Solution is equivalent to 1,250 pg/well.
 - d. Repeat the serial dilution. Add 1.5 mL of Buffer solution A to 0.5 mL of Calibrator Solution 1,250 pg/well to make a Calibrator Solution of 313 pg/well. Add 1.5 mL of Buffer solution A to 0.5 mL of Calibrator Solution at 313 pg/well to make a Calibrator Solution of 78 pg/well. Add 1.5 mL of Buffer solution A to 0.5 mL of Calibrator solution at 78 pg/well to make a Calibrator Solution at 19.5 pg/well. Add 1.5 mL of Buffer Solution A to 0.5 mL of Calibrator Solution at 19.5 pg/well to make a Calibrator Solution at 4.9 pg/well.
 - e. Transfer 1 mL of Buffer solution A to a test tube and use as Calibrator Solution 0 pg/well.
 - f. Store prepared Calibrator Solutions frozen.
2. Bradykinin Antibody Solution:
Add exactly 15 mL of purified water to the vial of Bradykinin Antibody Concentrate and dissolve the contents completely. Diluted Bradykinin Antibody Solution is stable for at least 1 week when stored frozen.
3. Wash Buffer: Transfer the entire volume of Wash buffer Concentrate (both 30 mL bottles) to a 500 mL graduated cylinder and add purified water to make a total volume of 300 mL. Store diluted Wash Buffer Solution at 4°C for up to 1 week.
4. Buffer Solution C: Add 1.5 mL of purified water to 0.3 mL of Deproteinizing Reagent in a test tube, mix, then add 1.8 mL of Buffer Solution B and mix to prepare Buffer Solution C.
5. Bradykinin Enzyme Conjugate Solution: Add exactly 8 mL of purified water to the vial of Bradykinin Enzyme Conjugate and dissolve the contents completely. Store diluted Bradykinin Enzyme Conjugate Solution at 4°C for up to 1 week.
6. Substrate Solution: Add one Substrate Tablet to one bottle of Substrate Diluent Buffer and dissolve the tablet to prepare the Substrate Solution. Prepare immediately before use and protect from light after preparation. Discard the remaining Substrate Solution after use, since storage of the dissolved Substrate Solution is not recommended.

STORAGE

Store kit at 4°C. Protect from light.

SPECIMEN COLLECTION AND HANDLING

1. Urine Samples:
Transfer 500 μ L of a urine sample to a plastic tube, add 100 μ L of Deproteinizing Reagent, mix, then centrifuge at 3,000 rpm (1,000 - 1,500 x g) for 10 minutes at 4°C. Transfer 250 μ L of the resulting supernatant into another plastic tube, then add 250 μ L of Buffer Solution B and mix to prepare the pretreatment sample.
2. Blood Samples:
Collect 5 mL of venous blood from the medial cubital vein or arterial blood from the brachial artery with a plastic syringe without anti-coagulant, and immediately (within 10 seconds) add the collected blood to a plastic tube containing 20 mL of ice-cooled absolute ethanol (HPLC grade) after removal of needle from the plastic syringe and mix well by shaking for 1 minute. Centrifuge at 1,500 x g at 4°C for 30 minutes and collect the supernatant. Re-extract the pellet with 5 mL of 80% ethanol and collect the supernatant after centrifugation. Evaporate the collected sample supernatant (extracts) under reduced pressure, and add 1 mL of distilled water to dissolve the residue. Adjust the pH of this solution to 2-3 with 0.1 N HCl, and wash the acidified solution twice with 3 mL of diethyl ether to remove sample lipids. After removal of diethyl ether by aspiration, evaporate the water phase under reduced pressure until dry. Dissolve the residue in an appropriate volume of Buffer Solution C, then centrifuge at 10,000 x g at 4°C for 30 minutes. Use the supernatant as a sample for the assay.

ASSAY PROTOCOL

- Duplicate determinations of Calibrator Solutions are recommended.
 - Allow the wells and the reagents to come to room temperature before starting assay.
 - Keep the plate in a horizontal position during the reaction.
1. Remove the desired number of Anti-Rabbit IgG-Coated Wells for determination, pipette 100 μL of prepared Bradykinin Antibody Solution into these wells, and mix the solution with a plate rotator. Incubate at room temperature for 1 hour, then aspirate the reaction mixture and wash the wells with 300 μL of diluted Wash Buffer with a plate washer. Repeat this procedure three times. Immediately proceed to the next procedure without drying the wells.
 2. Add 100 μL of Buffer Solution C and 50 μL of each prepared Calibrator Solution to predetermined wells (to be used for the calibration curve). Add 50 μL of Buffer solution A and 100 μL of pretreatment sample to predetermined reaction wells. Mix using a plate rotator, and incubate at room temperature for 1 hour.
 3. Add 50 μL of Bradykinin Enzyme Conjugate Solution to each well, mix with a plate rotator. Incubate overnight at 4°C.
 4. Remove the reaction mixture with a plate washer and wash each well with 300 μL of diluted Wash Buffer. Repeat this procedure four times. After washing, remove the residue of solution by turning the plate upside down and tapping on a paper towel. Never dry the wells completely.
 5. Add 100 μL of Substrate solution to each well and allow to incubate at room temperature for 30 minutes. (During this step, protect from light, for example by wrapping the plate with aluminum foil.)
 6. To stop the enzymatic reaction, add 100 μL of Stop Solution.
 7. Stir with a plate rotator.
 8. Measure the absorbance of each well at 492 nm with a plate reader. If possible, measure at two wavelengths (i.e. primary wavelength of 492 nm and a reference wavelength of 620 nm).

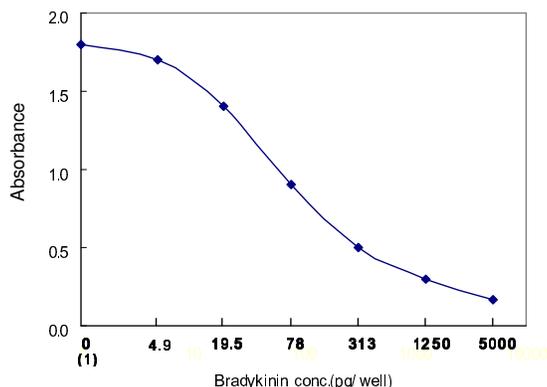
RESULTS

1. Plot the absorbance of each Calibrator Solution on the ordinate (uniform scale) against the concentration of each Calibrator Solution on the abscissa (logarithmic scale) and draw a smooth curve nearly fitting the points.
2. Using the calibration curve obtained, read the Bradykinin concentration corresponding to the absorbance of a pretreated sample. The Bradykinin concentration is expressed as the mass (pg) of Bradykinin per well in 100 μL of pretreated sample (unit: pg/well).
3. To calculate the Bradykinin concentration (pg/mL) of the original samples, multiply the reading values as expressed in pg/well by the following coefficient:
 - a. Urine Sample: 24
 - b. Blood Sample: (volume of Buffer Solution C / volume of the treated blood sample) x 10

For high-concentration samples (more than 5,000 pg/well), it is necessary to dilute the pretreated sample with Buffer Solution C appropriately or to dilute the original sample with purified water appropriately before pretreatment, and then to perform the procedure and subsequent procedures, and to convert by multiplying the reading by the dilution factor. Do not dilute the pretreated sample with purified water for determination.

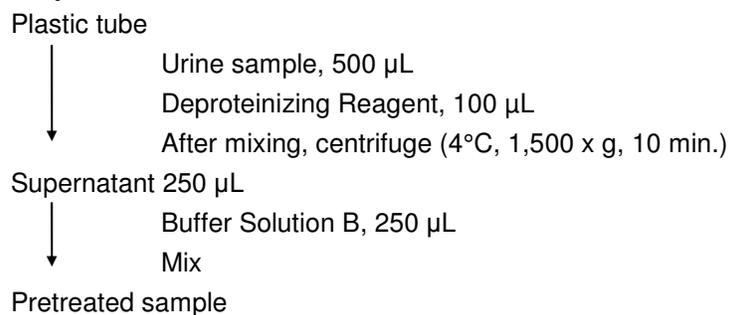
PERFORMANCE

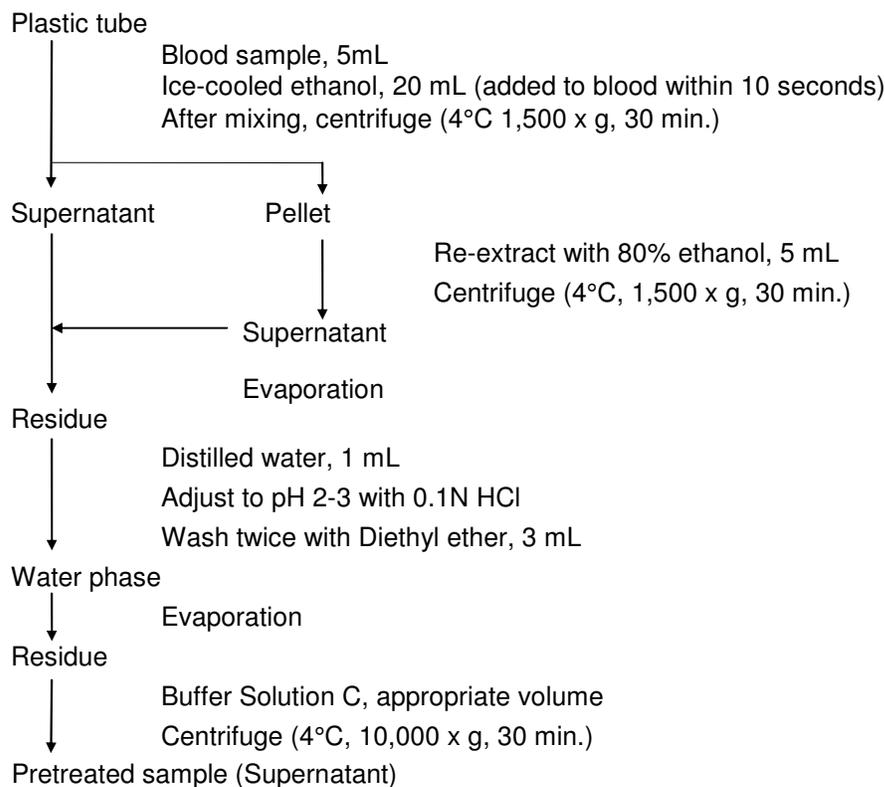
Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Cross-Reactivity

Compounds	%
Bradykinin	100
Lys- Bradykinin	100
Met-Lys- Bradykinin	100
T-Kinin	100
Tyr- Bradykinin	100
(Hyp ³)- Bradykinin	79
Des-Arg ¹ - Bradykinin	45
Des-Arg ⁹ -(Leu ⁸)- Bradykinin	< 0.1
[1-8]-Bradykinin	0.2
[1-7]-Bradykinin	< 0.1
[1-6]-Bradykinin	< 0.1
[1-5]-Bradykinin	< 0.1
BK potentiator B	< 0.1
BK potentiator C	< 0.1
Angiotensin I	< 0.1
Angiotensin II	< 0.1
LMW-Kininogen	< 0.1
Kininogen (Bovine Plasma)	< 0.1

PROTOCOL SUMMARY**Pretreatment Procedure for Samples****Urine Samples**

Blood Sample**Assay Procedure**

1) Preparation of Reaction wells

Anti-rabbit IgG coated well: Bradykinin Antibody Solution, 100 μ L

After mixing, incubate at room temperature for 1 hour

Wash the well three times with 300 μ L of diluted Wash Buffer

Reaction well

2) Assay method

	(For preparing calibration curve)	(For determining sample)
Reaction well	1 well	1 well
Buffer Solution C	100 μ L	—
Calibrator Solution	50 μ L	—
Buffer Solution A	—	50 μ L
Pretreated sample	—	100 μ L

After mixing, incubate for 1 hour at room temperature (primary antigen-antibody reaction)

Bradykinin Enzyme Conjugate Solution 50 μ L

After mixing, incubate overnight at 4°C (secondary antigen-antibody reaction)

Wash each well four times with 300 μ L of diluted Wash buffer

Substrate Solution 100 μ L

Incubate at room temperature for 30 minutes (enzymatic reaction)

Stop Solution 100 μ L

Measure the absorbance at 492 nm (if possible, use a secondary λ of 620 nm)

FOR RESEARCH USE ONLY

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