Bradykinin ELISA Kit
Catalog #IBRDKT

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 precur-sor 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 kal-likrein system in blood (related to the blood clotting cascade) but can also be gener-ated in other tissues and organs.
Besides kallikrein, other proteases such as plasmin may also release bradykinin. Several peptidases can degrade kinins, including Angio-tensin 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

▪ Introduction

Kallidinogenase, as a cardiovascular active substance, is one of the kininogenases which releases kinins by specifically acting on kininogens. It has been widely used in medical treatment as a cardiovascular drug, such as an antihypertensive drug. It is generally acknowledged that the measurement of kinin-degrading enzymes (kininases) and kinin-releasing activity in the bulk substance or preparations is important in the process and quality control in the manufacture of kalidinogenase preparations. Kininases are determined by a bioassay method with bradykinin (BK) as a substrate using the rat uterus or guinea pig ileum. In contrast, for the measurement of kinin-releasing activity, a bioassay method is used in which the formed kinin is determined using kininogen as a substrate. However, these bioassay methods are complicated in procedure and require a great deal of labor. Therefore, the Bradykinin ELISA kit has been developed as an enzyme linked immunosorbent assay (ELISA).

▪ Characteristics

1) The Bradykinin ELISA is a kit for the determination of BK is an ELISA using microstrip wells.
2) The Bradykinin ELISA permits a more convenient assay using a common ELISA instrument system (microplate reader, microplate washer, etc).
3) The Bradykinin ELISA is highly efficient in determining multiple samples.
4) The sensitivity (lower limit of detection) of the Bradykinin ELISA is 0.173 ng/ml (7.2 pg/well).
5) The Bradykinin ELISA can determine BK concentration in blood and urine samples specifically and accurately.

▪ Contents

Each kit (96 determinations) contains the following reagents:

Standard (Lyophilized) One vial contains: 100 ng Bradykinin	1 vial (for 1 ml)
Buffer solution A	1 bottle (30 ml)
Deproteinizing reagent Each ml contains: 200 mg Trichloroacetic acid	1 bottle (15 ml)
Buffer solution B	1 bottle (30 ml)
Antibody (Lyophilized) One vial contains: 0.15 ml anti-Bradykinin antibody (rabbit) concentrate	1 vial (For 15 ml)
Anti-rabbit IgG coated wells 12 x 8 microtiter strips with anti-rabbit IgG antibody (goat) precoated on each well	1 plate (96 wells)
Wash buffer concentrate	2 bottles (30 ml each)
Enzyme conjugate (Lyophilized) One vial contains: 0.08 ml Peroxidase (horseradish) labeled Bradykinin concentrate	1 vial (For 8 ml)
Substrate tablet Each tablet contains: 13 mg O-Phenylenediamine dihydrochloride	1 pack (2 tablets)
Substrate diluent buffer Each bottle contains: 9.75% Hydrogen peroxide	2 bottles (15 ml each)
Stop reagent	1 bottle (15 ml)

Two sheets of graph paper are included for preparing standard curves.

▪ Application

Determination of Bradykinin (BK) in urine and plasma

▪ Principle

1) BK in a sample and peroxidase labeled BK are allowed to react competitively to anti-BK antibody (rabbit) captured by anti-rabbit IgG antibody (goat) coated on microstrip wells.
2) The BK concentration is determined from the enzyme activity of peroxidase labeled BK bound to anti-BK antibody.

▪ Assay Method

1. Instruments and equipment required but not provided

Pipettes, Multichannel pipettes and reservoirs, measuring pipettes (1-10 ml), graduated cylinder (500 ml), refrigerated centrifuge (4°C, centrifugal force: 1,000 -1,500 x g), microplate mixer, microplate reader (492 nm primary wavelength, 620 nm reference wavelength), microplate washer.

For blood sample pretreatment: HPLC absolute ethanol (ice-cooled), plastic syringes, 0.1 N HCl, diethyl ether, refrigerated centrifuge (4°C, 10,000 x g), vacuum system.

Other: Plastic tubes for sample pretreatment, timer, Paper towels, Aluminum foil, etc.

2. Preparation of reagents

(1) Standard solutions

1) Provide seven test tubes.
2) Place exactly 1.0 ml of purified water in the Standard vial, mix gently to dissolve the contents completely, and transfer into a test tube to use as Standard solution 5,000 (pg/well).
3) Add 1.5 ml of Buffer solution A to a test tube, add 0.5 ml of Standard solution 5,000 (pg/well); then mix to prepare Standard solution 1,250 (pg/well).
4) Following similar dilution procedures, prepare:

   Standard solution 313 (pg/well): Add 0.5 ml of Standard solution 1,250 to 1.5 ml of Buffer solution A;
   Standard solution 78 (pg/well): Add 0.5 ml of Standard solution 313 to 1.5 ml of Buffer solution A and;
   Standard solution 19.5 (pg/well): Add 0.5 ml of Standard solution 78 to 1.5 ml of Buffer solution A and;
   Standard solution 4.9 (pg/well): Add 0.5 ml of Standard solution 19.5 to 1.5 ml of Buffer solution A.
5) Add 1 ml of Buffer solution A to a test tube, and use it as Standard solution 0.
6) Freeze Standard solutions when storing.

(2) Antibody solution

Add exactly 15 ml of purified water to the Antibody vial and dissolve the contents completely. (This Antibody solution is stable for at least 1 week when stored frozen.)

(3) Wash buffer

Transfer 60 ml of the Wash buffer concentrate (both 30 ml bottles) to a 500 ml graduated cylinder and add purified water to bring the volume to 300 ml. (Store this diluted Wash buffer in a refrigerator and use within 1 week.)

(4) Buffer solution C

Add 1.5 ml of purified water and 0.3 ml of Deproteinizing reagent to a test tube, mix, and then add 1.8 ml of Buffer solution B and mix to yield Buffer solution C.

(5) Enzyme conjugate solution

Add exactly 8 ml of purified water to the Enzyme conjugate vial and dissolve the contents completely. (Store this Enzyme conjugate solution in a refrigerator and use within 1 week.)

(6) Substrate solution

Place one Substrate tablet in 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 any remaining solution after use. Do **not** store for later use.)

3. Pretreatment of samples

The following methods are recommended:

(1) Urine Sample

Transfer 500 µl of a urine sample to a plastic tube, add 100 µl of Deproteinizing reagent, and mix, then centrifuge at 3,000 rpm (1,000-1,500 x g) for 10 minutes at 4°C. Transfer 250 µl of the resulting supernatant into another plastic tube, add 250 µl of Buffer solution B, and then mix to prepare the pretreated sample.

(2) Blood Sample

Aspirate 5 ml of venous blood from median cubital vein or arterial blood from brachial artery with plastic syringe without anti-coagulant. Immediately (within 10 seconds) add this collected blood to a plastic tube containing 20 ml of ice-cooled absolute ethanol (HPLC grade). After removing the needle from the plastic syringe for disposal, mix well for 1 minute by shaking. 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. Pool the collected supernatants and then evaporate under reduced pressure (vacuum system), and add 1 ml of distilled water to resolve 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 the lipids contained in the sample. After removal of diethyl ether by aspiration, evaporate the water phase under reduced pressure to dryness (vacuum system). Dissolve the residue in appropriate volume of Buffer solution C (record volume), then centrifuge at 10,000 x g at 4°C for 30 minutes. 100 µl of this supernatant will be used in the BK assay.

4. Procedure

- Duplicate determinations of Standard solutions and samples are preferable.
- Allow the wells and the reagents to come to room temperature before starting assay.
- Keep the plate in the horizontal position during the reaction.

(1) Preparation of Reaction wells - Take out the necessary number of Anti-rabbit IgG coated wells for the determination, pipette 100 µl of Antibody solution into these wells, stir with a microplate mixer, then allow to stand at room temperature for 1 hour. Next, remove the reaction mixture with a microplate

washer and wash the wells with 300 µl of Wash buffer. Repeat this procedure for a total of 3 washes. Immediately proceed to the next procedure without drying the wells.

(2) Add 100 µl of Buffer solution C and 50 µl of each Standard solution to the standard curve wells. Add 50 µl of Buffer solution A and 100 µl of each pretreated sample to the sample determination wells. Stir with a microplate mixer, and then allow to stand at room temperature for 1 hour.

(3) Add 50 µl of Enzyme conjugate solution to each well, stir with a microplate mixer, and then allow to stand overnight at 4°C.

(4) Remove the reaction mixture with a Microplate washer and wash each well with 300 µl of Wash buffer. Repeat this procedure for a total of 4 washes. After washing, remove the residue of solution by putting 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 stand at room temperature for 30 minutes. (During this step, protect the wells from light, e.g, wrap the plate with aluminum foil.)

(6) To stop the enzymatic reaction, add 100 µl of Stop reagent to each well in the same time intervals as the Substrate solution was added.

(7) Stir with a microplate mixer.

(8) Measure the absorbance of each well at 492 nm with a microplate reader. (It is preferable to measure at two wavelengths, i.e., primary wavelength of 492 nm and reference wavelength of 620 nm.)

5. Outline of assay procedures

(1) Pretreatment procedure of samples

1) Urine sample

Plastic tube

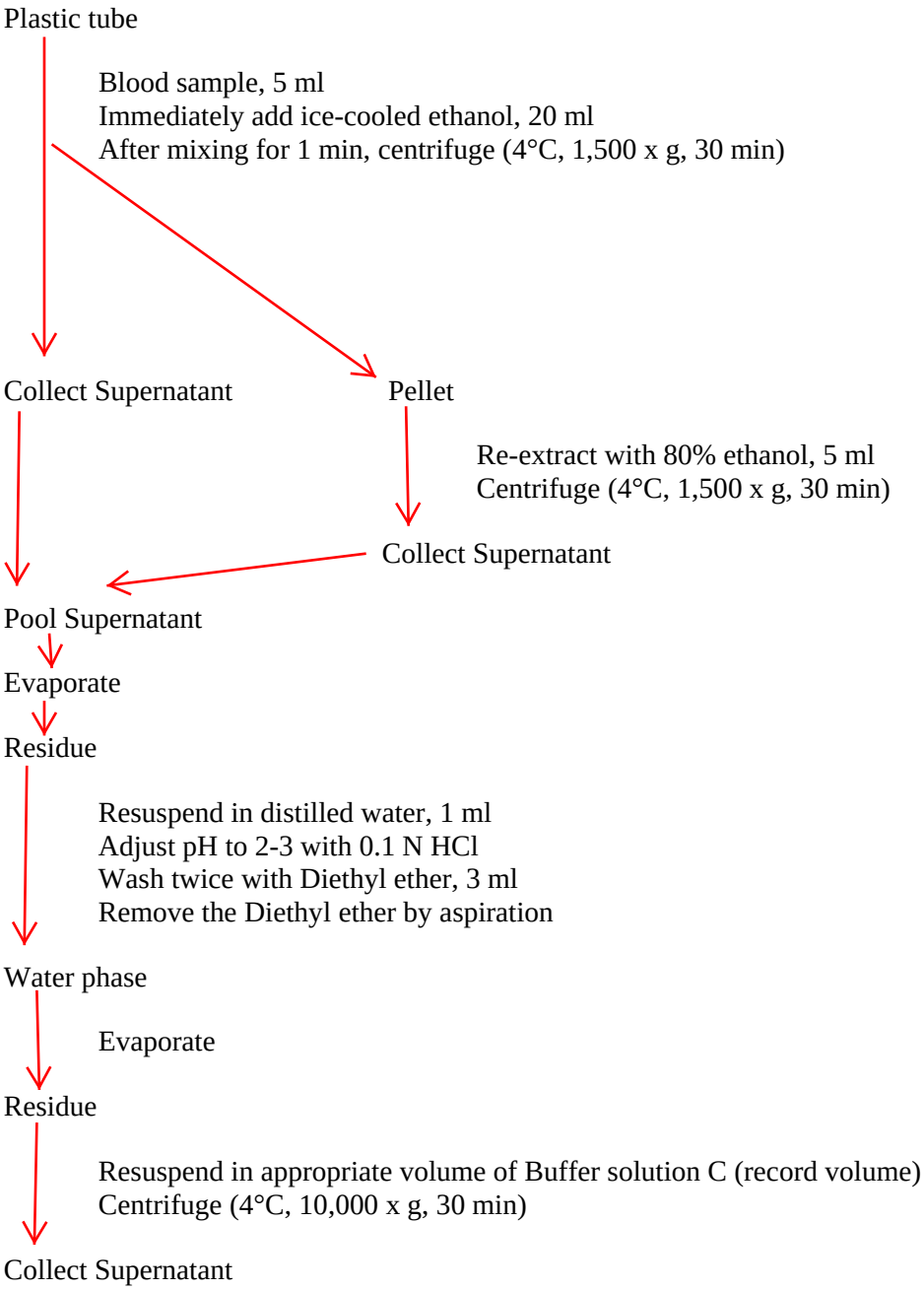
↓ Urine sample, 500 µl
Deproteinizing reagent, 100 µl
After mixing, centrifuge (4°C, 1,500 x g, 10 min)

Collect Supernatant, 250 µl

↓ Buffer solution B, 250 µl
Mix

100 µl of this pretreated sample will be used in the assay

2) Blood sample



100 µl of this pretreated sample will be used in the assay

(2) Assay procedure

1) Preparation of Reaction wells

Anti-rabbit IgG coated wells

↓ BK antibody solution, 100 µl

After stirring, allow to stand at room temperature for 1 hour

↓

Wash the wells three times with 300 µl of Wash buffer

↓

Reaction wells

2) Assay method

	(For preparing standard curve)	(For determining samples)
Reaction wells	1 well	1 well
Buffer solution C	100 µl	-
Standard solution	50 µl	-
Buffer solution A	-	50 µl
Pretreated sample	-	100 µl

After stirring, allow to stand for 1 hour at room temperature (primary antigen-antibody reaction)

Enzyme conjugate solution 50 µl

After stirring, allow to stand overnight at 4°C (secondary antigen-antibody reaction)

Wash each well four times with 300 µl of Wash buffer

Substrate solution 100 µl

Allow to stand at room temperature for 30 minutes, protected from light (enzymatic reaction)

Stop reagent 100 µl

Measure the absorbance at 492 nm (reference wavelength 620 nm)

6. Preparation of a standard curve and reading of BK concentration

(1) The abscissa (logarithmic scale) and the ordinate (uniform scale) of the graph paper (semilogarithmic paper) included with the kit represent the concentration of Standard solution and the absorbance, respectively. Plot the absorbance of each Standard solution and draw a smooth curve nearly fitting the points.

(2) By using the standard curve obtained, read the BK concentration corresponding to the absorbance of a pretreated sample. The BK concentration is expressed as the weight (pg) per well of BK in 100 µl

of the pretreated sample (unit: pg/well).

(3) To calculate the BK concentration (pg/ml) of the original samples, multiply the values read off the standard curve (expressed in pg/well) by the following coefficients:

1) Urine Sample: 24
2) Blood Sample: (volume of Buffer solution C / volume of the treated blood sample) x 10

(4) For high concentration (more than 5,000 pg/well) samples, it is necessary to:

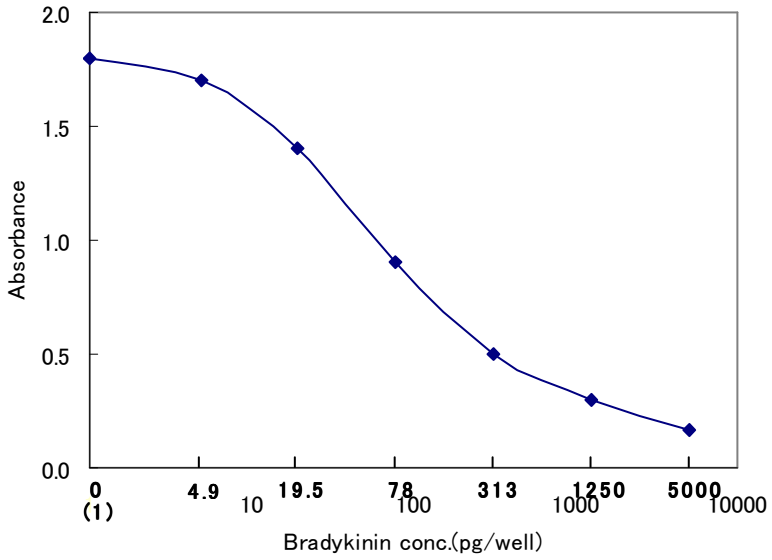
a) dilute the pretreated sample with Buffer solution C appropriately

or

b) dilute the original sample with purified water appropriately and then follow the pretreatment procedure
(Do *not* pretreat the sample first, and then dilute with purified water.)

The resulting sample reading must then be multiplied by the appropriate dilution factor.

Typical Standard Curve



▪ Cross-reactivity

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

▪ **Precautions for use or in handling**

1. General precautions

(1) In order to obtain reliable and consistent results, the instructions in this booklet must be strictly adhered to.
(2) Do not use the kit reagents after the date of expiry.
(3) Do not mix the enzyme conjugate, the Antibody, or the Anti-rabbit IgG coated wells from different lots of kits.
(4) White powder may adhere on the Anti-rabbit IgG coated wells. This will not influence the results.
(5) Be sure to handle the Deproteinizing reagent carefully because its main ingredient, trichloroacetic acid, is very corrosive to the skin.
(6) Be sure to handle the Stop reagent carefuly. It contains sulfuric acid.
(7) If the kit reagents come into contact with skin, mouth, or eyes, irrigate with water and seek medical attention immediately.
(8) Read the instructions of the instruments or equipment required for the assay carefully.
(9) Use the kit carefully under the supervision of personnel who have appropriate knowledge of safety regarding biological research and experiments.

2. Procedure cautions

(1) It is preferable to determine samples in duplicate until you are accustomed to the procedure.
(2) Add the reagents in the order as directed in the Assay method. Proceed with samples and Standard solutions simultaneously, under the same conditions.
(3) When two or more Substrate tablets are used simultaneously, dissolve each tablet separately in each bottle of Substrate diluent buffer, then mix in another container before use.
(4) Do not damage or dirty the bottom of the Anti-rabbit IgG coated wells. The wells act as cuvettes to measure the absorbance.
(5) Do not foam or spatter the working solution in the wells in order to prevent contamination between the wells.
(6) Avoid contamination between the kit reagents and the samples.

3. Safety cautions

(1) Virus:

As no human serum or plasma is used in this kit, the kit reagents have little risk of causing infection of HBV, HIV, or HCV. However, the kit reagents should be handled with the same precautions at any potentially biohazardous materials.

Inactivate virus in samples, reagents, and used apparatus by one of the following methods when the assay is completed.

a) Autoclave (121°C, 20 min/ 115°C , 30 min)
b) Soak in sodium hypochlorite (available chlorine at 25,000 ppm for 30 min to 1 hr/ at 10,000 ppm overnight)
c) Soak in glutaraldehyde (2% for over 1 hr)

(2) Pipetting:

Do **not** pipette sample or reagents by mouth.

4. Cautions for waste

The assay waste solution should be treated by the same methods as shown above, section 3.(1), and then diluted with large quantities of water if disposing of in the sink.

▪ Storage and expiry period

Storage: Store in a cool place (2-10°C) and protect from light
Expiry period: 1 year (indicated on the box label)