Bradykinin Assay Kit-IBL 96Well

Please read carefully this instruction prior you use this assay kit.
This product is for research use only. It shall not be used for any other purposes rather than research use.

【Kit Component】

Bradykinin Assay Kit 1 kit [96Well (including for standard curves)] consists of the following reagents.

	Reagent	Quantity	Description
1.	Antibody-2 binding plate	: 1 plate (96 wells)	Anti-rabbit IgG antibody binding plate (8 well strip x 12 rows)
2.	Labeled antigen (lyophilized)	: 1 bottle (8 mL)	Peroxidase (derived from horseradish) Labeled bradykinin
3.	Standard reagent (lyophilized)	: 1 bottle (1 mL)	Bradykinin 100 ng
4.	Antibody-1 (lyophilized)	: 1 bottle (15 mL)	Anti-bradykinin antibody
5.	Buffer solution A	: 1 bottle (30 mL)	
6.	Buffer solution B	: 1 bottle (30 mL)	
7.	Deproteinizer	: 1 bottle (15 mL)	200 mg of trichloroacetic acid in 1 mL
8.	Substrate	: 1 bag (2 tablets)	o-phenylenediamine dihydrochloride
9.	Substrate buffer	: 2 bottles (15 mL each)	
10.	Stop solution	: 1 bottle (15 mL)	
11.	Wash buffer conc.	: 1 bottle (60 mL)	

【Purpose of use】

Measurement of bradykinin

【Principle】

Bradykinin Assay Kit is an assay kit based on a competitive Enzyme-linked immuno-sorbent assay (ELISA) using a microstrip plate. Bradykinin concentration is measured from enzymatic activity of peroxidase which is bound to a microstrip plate by competitive reaction of bradykinin in samples and peroxidase-labeled bradykinin against to an antibody-1 [anti-bradykinin antibody] which is captured an antibody-2 [anti-rabbit IgG antibody] coated on a microstrip plate.

【Method of operation】

1. Required Equipment or machines
   Automated pipettes, multi-channel pipettes, reservoirs for multi-channel pipettes, measuring pipettes (1 to 10 mL), measuring cylinders (500 mL), centrifuge (for multiple racks, having a centrifugal capacity of 1,500xg or more), Microplate mixer, microplate reader, microplate washer.
   Others: Test tubes (plastic), test tubes (glass), a stop watch, paper towels, aluminum foil, graph paper (single-log), and other cooling centrifuges for pretreatment of blood samples (with a centrifuge capacity of 10,000xg or more) etc.

2. Preparation of Reagent
   (1) Preparation of standard solution
      1) Prepare 7 test tubes (plastic).
      2) Accurately add 1.0 mL of purified water to the bottle of standard reagent, mix gently to completely dissolve the contents, and transfer to a test tube to make the standard solution (5,000 pg/well).
      3) Take 1.5 mL of buffer solution A in a test tube, add 0.5 mL of standard solution (5,000 pg/well), and mix to prepare standard solution (1,250 pg/well).
      4) In the same way, serially dilute the solution prepared at 3) by 4-fold.
         - Add 0.5mL of standard solution (1,250 pg/well) into buffer solution A 1.5 mL to make standard solution (313 pg/well).
         - Add 0.5mL of standard solution (313 pg/well) into buffer solution A 1.5 mL to make standard solution (78 pg/well).
         - Add 0.5mL of standard solution (78 pg/well) into buffer solution A 1.5 mL to make standard solution (19.5 pg/well).
         - Add 0.5mL of standard solution (19.5 pg/well) into buffer solution A 1.5 mL to make standard solution (4.9 pg/well).
      5) Add 1 mL of buffer solution A in a test tube to make standard solution 0.
      6) The standard solution prepared above should be sealed and kept as frozen.
   (2) Preparation of antibody-1 solution
      Accurately add 15 mL of purified water into the bottle of antibody-1 to reconstitute. (The reconstituted antibody-1 should be stored as frozen with the lid closed and it should be used within 1 week.)
   (3) Antibody-2 binding plate
      Prepare the required number of antibody-2 binding plates for measurement. Remove unused strips from the frame, and return it to the original bag, then, seal and store in a refrigerator.
   (4) Adjustment of washing solution
      Scale 30 mL undiluted washing solution into a 500 mL size cylinder, and add purified water to make 300 mL. (This washing solution should be sealed, kept at a refrigerator, and it should be used within 1 week.)
   (5) Prepration of buffer solution C
      Add 1.5 mL purified water and 0.3 mL Deproteinizer into a test tube (glass) and mix well, then add 1.8 mL buffer solution B and mix to make buffer solution C.

(6) Preparation of labeled antigen solution
Accurately add 8 mL purified water into the bottle of labeled antigen to reconstitute. (The reconstituted labeled antigen solution should be stored in a refrigerator with the lid closed and it should be used within 1 week.)

(7) Preparation of substrate solution
Add a substrate tablet to one bottle of the substrate solution and dissolve it to prepare a substrate solution. Preparation is immediately done before use and shield it from light after the preparation. (Dispose the residual liquid after use. It cannot be reused.)

3. Sample pretreatment

The following pretreatment methods are recommended.

(1) In the case of urine samples (and non-whole blood samples)
After take 500 μL urine sample, add 100 μL a Deproteinizer in a plastic tube and mix it, centrifuge at 4 ℃, 1,500xg for 10 minutes. Place 250 μL the supernatant in another plastic tube, and add 250 μL buffer B to be mixed, then it to be pretreated urine sample. Plasma samples and tissue culture solution can be processed in the same manner.

(2) In the case of blood (whole blood)
Collect 5 mL of arterial or venous blood using a plastic syringe without anticoagulant and immediately (within 10 seconds) add it to a polypropylene tube with a lid which containing 20 mL 100 v / v% ice-cold ethanol (for HPLC), and mix it well for 1 minute. After centrifuge at 4 ℃, 1,500xg for 30 minutes, collect the supernatant. The sediment is together re-extracted with 5 mL of 80v / v% ethanol and after centrifuged in the same manner, the supernatant is dried under reduced pressure. After dissolving the dried one in 1 mL purified water, adjust the pH to 2-3 with 0.1 mol/L HCl. After washing this solution twice with 3 mL of diethyl ether, the aqueous layer is dried under reduced pressure. Dissolve the dried one in an appropriate amount of buffer C and centrifuge at 4 ℃, 10,000xg for 30 minutes. The supernatant is collected in a plastic tube and used as a pretreated blood sample.

4. Operation method

- Measurement in duplicate is desirable for standard solutions.
- Return the plates and reagents to room temperature before use.
- Make sure to keep it horizontal during reaction in wells.

(1) Prepare required number of antibody-2 binding plates for measurement, dispense 100 μL antibody-1 solution each, and after mix it with a microplate mixer, leave it to stand at room temperature for 1 hour. After 1 hour, remove the reacted solution using a microplate washer, and add 300 μL washing solution to each well for washing. Repeat this operation 3 times. Do not dry the wells and immediately move on to the next operation. (Preparation of reaction wells)

(2) Add 100 μL buffer C and 50 μL standard solution for creating a standard curve, and 50 μL buffer A and 100 μL pretreated sample for sample measurement to the reaction wells. Then, after mixing using a microplate mixer, leave it to stand at room temperature for 1 hour.

(3) Add 50 μL labeled antigen solution to each well, and after mixing it using a microplate mixer, leave it to stand at 4 ℃ overnight.

(4) Remove the reacted solution using a microplate washer and add 300 μL washing solution to each well for washing. Repeat this operation 4 times. After washing, tap the plate upside down on a paper towel to remove excess liquid. (However, do not allow the wells to be completely dried.)

(5) Add 100 μL substrate solution to each well and leave it to stand at room temperature for 30 minutes. (At this time, wrap the plate with aluminum foil to block light.)

(6) Add 100 μL stop solution to each well and mixing it using a microplate mixer.

(7) Measure the absorbance of each well at 492 nm (preferably two-wavelength measurement at the main wavelength of 492 nm and the sub-wavelength of 620 nm).

5. Outline of measurement operation

(1) Sample pretreatment

1) Urine sample pretreatment

Plastic tube
Urine sample 500μL
Deproteinizer 100μL
↓ Centrifuge after mixing (4 ℃, 1,500×g, 10 minutes)
Supernatant 250μL
Buffer solution B 250μL
↓
Pretreated urine sample

2) Blood sample pretreatment

Plastic tube
Blood sample 5mL
Ice cold ethanol 20mL
↓Centrifuge after mixing (4 ℃, 1,500 xg, 30 minutes)
Supernatant collection
Re-extract sediment with 80v / v% ethanol 5mL
↓ Centrifuge (4 ℃, 1,500xg, 30 minutes)
Combine the supernatants and dry
↓
Dissolved in purified water 1mL
Adjusted pH to pH2-3 with 0.1 mol/L HCl
↓
Washed with diethyl ether 3 mL x 2 times
↓
Dry the water layer
↓
Dissolved in buffer solution C, appropriate amount
↓
Centrifuge (4 ℃, 1,500xg, 30 minutes)
↓
Supernatant
↓
Pretreated blood sample

(2) Measurement operation

1) Preparation of reaction wells

Antibody-2 binding plate
↓ Antibody-1 solution 100 µL
After mixing, leave it to stand at room temperature for 1 hour
↓
Wash the wells 3 times with washing solution 300 µL x 3 times
↓
Reaction well

2) Measurement method

	For standard Curve creation	For sample measurement
Reaction well	1 well	1well
Buffer solution C	100uL	-
Standard solution	50uL	-
Buffer solution A	-	50uL
Pretreated sample	-	100uL

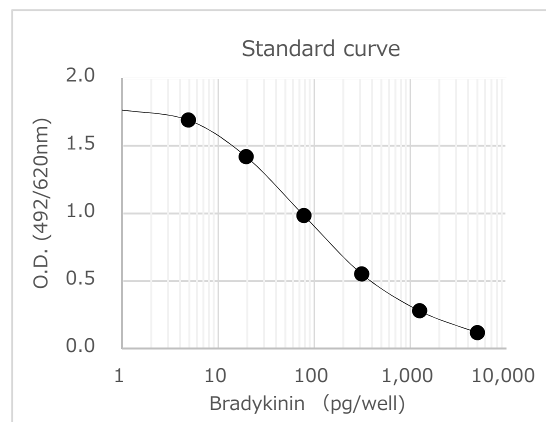
After mixing, leave it to stand at room temperature for 1 hour
Labeled antigen solution 50uL
↓After mixing, leave it to stand at 4 ℃ overnight
Wash the wells 4 times with washing solution 300uL x 4 times
↓
Substrate solution 100uL
↓Leave it to stand at room temperature for 30 minutes
Stop solution 100uL
↓
Measure the absorbance at a wavelength of 492 nm
(Sub-wavelength 620nm)

6. Creating a standard curve and reading bradykinin concentration

(1) Using the obtained standard curve, read the bradykinin concentration corresponding to the absorbance of the pretreated sample. The bradykinin concentration is expressed as the amount of bradykinin in 100 µL of the pretreated sample used for the measurement as the weight (pg) per well. (Unit: pg/well)

(2) When determining the bradykinin concentration (pg/mL) per 1 mL of the original sample

1) For urine samples, multiply the reading in (1) by 24.
2) For blood samples, multiply the reading in (1) by (buffer C usage / blood sampling) x 10.
For example, when 5 mL of blood is pretreated and the final 0.5 mL buffer solution C is used as the pretreated sample,
(0.5/5)x10 = 1 will be multiplied, and the reading value pg/well in (1) will be pg/mL as is.
3) For high-concentration (bradykinin 5,000 pg/well or higher) samples, either diluting the pretreated sample appropriately with buffer C, or after diluting the original samples appropriately with purified water, and then, perform all operations after the pretreatment operation., 4. Operation method (1), then, it is necessary to calculate by multiplying dilution factors on the obtained reading values. The diluted pretreated samples with purified water should not be used for measurement.



【Cross-reactivity】

In addition to bradykinin, it is cross related to Lys-BK and Met-Lys-BK (100%), T-kinin (89%), Tyr-BK (91%), [Hyp3] –BK (31%)and Des-Arg1-BK (43%). It is hardly cross reacted to any metabolites of bradykinin.

Substances	Cross-Reactivity(%)	Substances	Cross-Reactivity(%)	Substances	Cross-Reactivity(%)
Bradykinin	100	Des-Arg1-BK	43	BK-Potentiator B	<0.1
Lys-BK	100	(1-8)-BK	0.1	BK-Potentiator C	<0.1
Met-Lys-BK	100	(1-7)-BK	<0.1	Angiotensin I	<0.1
T-Kinin	89	Des-Arg^9-[Leu^8]-BK	<0.1	Angiotensin II	<0.1
Tyr-BK	91	(1-6)-BK	<0.1	LMW-Kininogen	0.7
[Hyp^3]-BK	31	(1-5)-BK	<0.1	Kininogen (Bovine Plasma)	<0.1

【Precautions for use or handling】

1. Precautions for use
   (1) General precautions
   1) Do not use this kit for any other purposes rather than the methods and purposes described in the instruction manual.
   2) Do not use any reagent beyond the expiration date.
   3) Do not mix or replace the reagents with the reagents from a different lot or kit.
   4) White powder may be found on the surface of walls of the antibody-2 binding plate, however, it does not affect to measurement result.
   5) Be careful with handling of deproteinizer as the main active substance, trichloroacetic is highly corrosive against to the skin.
   6) Be careful with handling of stop solution as sulfuric acid is contained.
   7) If any reagent accidentally gets into your eyes or mouth, take first-aid measures such as rinsing thoroughly with water, and get medical treatment if necessary.
   8) Carefully read instruction manuals of the device or equipment to be used.
   9) Use this kit safely under the supervision of experts or supervisors who have enough knowledge of safety of biochemistry, pysics and chemistry experiments and research.
   (2) Precautions regarding operation method
   1) It is desirable to perform measurement of the sample in duplicate if you are not familiar with the operations.
   2) Follow the procedure for adding reagents as described in this instruction for use. Also, perform the sample and standard solution at the same time under the same conditions.
   3) Do not scratch or stain the bottom of the plate. (The well also serves as a cuvette for measuring absorbance.)
   4) To prevent contamination between wells, prevent liquid from foaming or adhering to the surroundings.
   5) Avoid microbial contamination of specimens and reagents, and contamination between reagents.

2. Prevention of biohazard
   (1) Virus
   Since this kit is not made from human serum, it is considered that there is rare possibility of HBV, HIV and HCV infection from the kit, however, the handling should be in same manners as handling of patient serum which may be infected with virus.
   Samples, each reagent, and all instruments / tools which were used for the experiment should be treated by either method.
   1) Treat with 3% SDS (sodium dodecyl sulfate) for 5 minutes at 100 ℃.
   2) High-pressure sterilization in an autoclave for 1 hour at 132 ℃.
   3) Immerse in 1 mol / L sodium hydroxide solution for 1 hour at room temperature
   4) Immerse in 1-5w / v% sodium hypochlorite for 2 hours at room temperature
   (2) Pipetting
   When pipetting each reagent or sample used for the test, do not suck it by mouth under any circumstances.

3. Precautions regarding disposal
   After the measurement is completed, any waste liquid should be treated according to the section 2. (1) Virus in the previous section, and then should be disposed with a sufficient amount of water.

【Storage / Shelf Life】

Storage : Avoid light and stored in chilled (2-8 ℃)

Shelf Life : The expiry date is specified on the outer box.

【Packaging unit and product number】

Bradykinin Assay Kit-IBL 1 Kit (96Well)
Product number 27790

【Name and address of Distributor】

Inagaki Chemicals Co., Ltd.
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