KAMIYA BIOMEDICAL COMPANY

Human PIVKA-II ELISA

For the quantitative determination of PIVKA-II in human plasma or serum

Cat. No. KT-694

For Research Use Only.

PRODUCT INFORMATION
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PRODUCT
The ***K-ASSAY***® Human PIVKA-II ELISA is an enzyme immunoassay for the quantitative determination of PIVKA-II in human plasma or serum. For research use only. PIVKA-Ⅱ is an acronym for Protein Induced by Vitamin K Absence or Antagonist. PIVKA-Ⅱ is equal to DCP(Des-γ-Carboxy-Prothrombin).

PRINCIPLE
This kit is a solid-phase(micro-cup)sandwich type EIA. In briefly, using anti-PIVKA-Ⅱ monoclonal antibody coated on the inner surface of the micro-cup, PIVKA-Ⅱ in the sample is captured and allowed to react with enzyme-labeled anti-human prothrombin antibody. When substrate solution is added to the reaction product, the enzyme reaction develops color. PIVKA-Ⅱ concentration of the sample is determined from the absorbance of the colored solution.

1. Reaction of PIVKA-Ⅱ with the solid-phase antibody(First Reaction)
When a sample is placed in the cup coated with anti-PIVKA-Ⅱ monoclonal antibody, an amount of PIVKA-Ⅱ binds to the solid-phase antibody coated on the cup in proportion to PIVKA-Ⅱ concentration of the sample.
2. Removal of the non-reactive enzyme-labeled antibody and coloration reaction(Second Reaction)
The inside of the cup is washed again to remove the non-reactive enzyme-labeled antibody, and enzyme-labeled anti-human prothrombin antibody is placed in the cup. The enzyme-labeled anti-human prothombin antibody as is bound to the PIVKA-Ⅱ forms a sandwich combination comprised of the solid-phase antibody, antigen(PIVKA-Ⅱ) and enzyme-labeled anti-prothrombin antibody.
3. Removal of the non-reactive enzyme-conjugated antibody and coloration reaction(Third Reaction)
The inside of the cup is washed again and the non-reactive enzyme-conjugated antibody is removed. When substrate solution is placed in the cup, enzyme-labeled antibody changes the enzyme substrate such that coloration occurs.
4.Calculation of the test results
Since the activity of enzyme bound to the solid-phase reflects the concentration of PIVKA-Ⅱ in the sample, the PIVKA-Ⅱ value can be calculated by measuring the absorbance of the colored solution and comparing it with that of the reference calibrator.

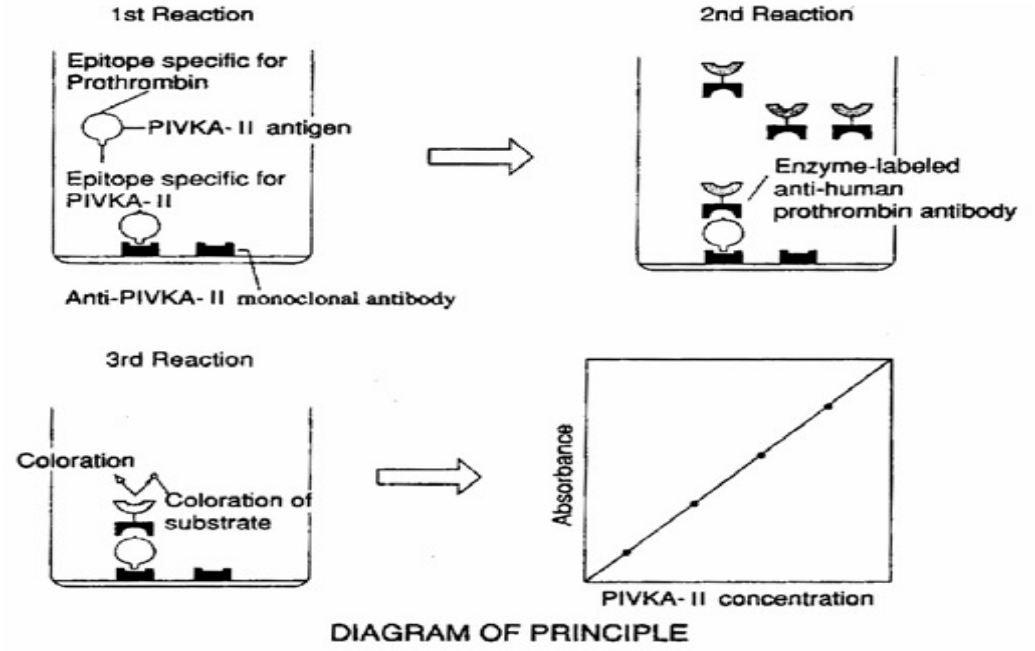

DIAGRAM OF PRINCIPLE

COMPONENTS

Reagent	Components	Quantity	No.
Standard antigen	Decarboxylated prothorombin(lyophilized)	10 ,25 ,500, 2000mAU/mL (dissolved soln.)	1 vial (each)
Sample diluent	Bovine albumin solution	3 mL/vial	1 vial
Antibody coated cup	Transparant polystyrene micro-cup coated with anti-PIVKA- Ⅱ monoclonal antibody(mouse)	(2×8 cups)×6 lines(96 cups)	1 package
Reaction solution	Pale yellow solution with 0.05mol/L tris-(hydroxymethyl)-aminomethane buffer contains normal rabbit serum(10%)	4 mL/vial	1 vial
Enzyme-antibody conjugate	Pale yellow solution with horseradish peroxidase-conjugated(labeled) anti-human prothrombin(rabbit), 10 ~ 40 E.U./mL	15 mL/vial	1 vial
Enzyme substrate	Oxydol(Japanese Pharmacopoeia)	0.5 mL/vial	1 vial
Chromogen	2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (lyophilized)	4 mL/vial (dissolved soln.)	3 vials
Stop-reaction solution	Clear and colorless solution with 2 mmol/L sodium azide	15 mL/vial	1 vial
Wash solution concentrate	0.9% sodium chloride solution with 1% polyoxyethylene-sorbitan monolaurate	10 mL/vial	1 vial

PROCEDURE

This kit is used to determine the PIVKA-Ⅱ concentration of the sample according to the principle of enzyme immunoassay.

1. Reagent preparation

(1) Standard antigen

To each vial of standard antigen of differing concentration add 1 mL of purified water (*) and dissolve. It is recommended that the reconstituted standards be freshly prepared for each assay.

(2) Substrate solution

To 1 vial of the chromogen(lyophilized) add 4 mL of purified water and dissolve. To the solution add 10 µL of enzyme substrate to obtain the substrate solution. Use the substrate solution immediately after preparation and do not preserve any for later use.

(3) Wash solution

To 300 mL of 0.9% sodium chloride solution (or saline solution) add 3 mL of washing solution concentrate per 32 cups. Do not preserve any leftover washing solution for later use.

(4) Reagents to be used as received

Use the sample diluent, antibody coated cups, enzyme-labeled antibody, reaction solution, and stop-reaction solution as they are.

(*)Purified water is water purified by distillation, ion-exchange treatment, ultrafiltration or combination of these methods.

２. Equipment and device required

(1) Cup holder

Metal holder (made of aluminum) designed to fit the periphery of an antibody-coated cup.

(2) Container

Container designed to prevent evaporation of water.

(3) Cup washing apparatus

Well-washer(e.g., Minilab washer)that automatically washes the cup by suction or an apparatus that supplies 250 µL of washing solution per cup and aspirator that removes the solution in the cup.

(4) Spectrophotometer

Microplate-autoreader

３. Procedure

(1) Take an antibody-coated cup out of the package and set in the cup holder.

(2) Add 25 µL of reaction solution to each cup.

(3) Add 100 µL of standard antigen solution of differing concentration and sample diluent(blank) to the duplicate cups.

(4) Add 100 µL of sample into each cup.

(5) Place the cups in the container and incubate for 16-24 hr. at 2-10℃(1st reaction).

(6) Discard the contents of each cup, and wash the cup with wash solution remove all solution from the cup.

(7) Add 100 µL of enzyme-conjugated antibody to all cups(2nd reaction).

(8) Place the cups in the container and incubate for 1 hr. at 20-30℃.

(9) Discard the contents of each cup, wash the cup with wash solution and remove all solution from the cup.

(10)Add 100 µL of substrate solution to all cups.

(11)Place the cups in the container and incubate at 20-30℃.

(12)10min. after the addition of substrate solution, measure the absorbance of sample diluent, standard antigen solution and sample at a plate reader wavelength of 405nm.(sub-wavelength of 492nm).(*)

(13)60min. after the addition of substrate solution, add 100 µL of stop-reaction solution to all cups to stop the reaction and measure the absorbance of sample diluent, standard antigen solution and sample at a plate reader wavelength of 405nm. (sub-wavelength of 492nm).(*)

(14)Using double logarithmic paper, draw a standard curve by plotting the values obtained by subtracting the absorbance(mean) of the blank from the absorbance(mean) of 500 and 2,000 mAU/mL of standard antigen solution determined 10 min. after substrate addition on the Y axis against the position of each standard antigen concentration on the X axis.(**)

(15)On the standard curve obtained from 10 min assay, locate the point corresponding to the values obtained by subtracting the absorbance(mean) of the blank from the absorbance(mean) of sample obtained from 10 min. assay and read off the corresponding PIVKA-Ⅱ concentration.

(16) Using double logarithmic paper, draw a standard curve by plotting the values obtained by subtracting the absorbance(mean) of the blank from the absorbance(mean) of 10 ,25 and 500 mAU/mL of standard antigen solution determined 60 min. after substrate addition on the Y axis against the position of each standard antigen concentration on the X axis.(**)

(17) On the standard curve obtained from 60 min. assay, locate the point corresponding to the values obtained by subtracting the absorbance(mean) of the blank from the absorbance of sample obtained from 60 min. assay and read off the corresponding PIVKA-Ⅱ concentration.

(*)For each batch(plate) test, the absorbance must be measured twice (after 10 and 60min.)for each cup and two type standard curve must be drawn to determine the PIVKA-Ⅱ concentration in the range of 10～2,000mAU/mL.

10mAU/mL≦sample≦500mAU/mL

(Use the standard curve obtained from 60min.assay)
501mAU/mL≦sample≦2,000mAU/mL

(Use the standard curve obtained from 10min.assay)
(**)It might be easier to calculate the PIVKA-Ⅱ concentration by using a plate reader calibration software.

PERFORMANCE CHARACTERISTICS

1. Sensitivity test
When the test was performed with 10 and 25 mAU/mL of standard antigen solution, the difference between the average absorbance values and that of sample diluent(0 mAU/mL) were not lower than 0.005 and 0.013,respectively,under the test conditions defined by manufacturer.
2. Specificity test
When the test was performed with control plasma, the PIVKA-Ⅱ concentration ranged from 80 to 120% of the known PIVKA-Ⅱ concentration under the conditions defined by the manufacturer.
3. Within run reproducibility
The coefficient of variation(C.V. value) was determined by performing 4 tests simultaneously each on 25 mAU/mL, 500 mAU/mL, and 2,000 mAU/mL of standard antigen solution, and the results were below 10% under the test conditions defined by the manufacturer.
4. Assay range
The assay range of this kit was 10-2,000 mAU/mL.
5. Effects of interfering substances
(1) Effects of hemoglobin
Hemoglobin did not at all affect the PIVKA-Ⅱ value at concentrations of up to 490 mg/dL.
(2) Effects of bilirubin
Free bilirubin did not at affect the PIVKA-Ⅱvalue at concentrations of up to 27 mg/dL. Such was also the case with conjugated bilirubin at concentrations of up to 22 mg/dL.
(3) Effects of Chyle
Chyle of up to 1,950 FTU (Formazin turbidity unit) did not at all affect the results of the test.

PRECAUTIONS

1. General precautions
(1) Observe the instructions for Test Procedure.
(2) The reagents in each kit are quality controlled as a unit. Do not interchange reagents from different lots.
(3) Although it has been ensured that the standard antigen used in this kit is negative for HBs antigen, anti-HCV antibody, and anti-HIV antibody, the standard antigen should be treated as potentially infectious.
(4)It is well to remember that PIVKA-Ⅱ concentration may decline when vitamin K preparations are used.
(5)It is also well to remember that PIVKA-Ⅱ concentration may rise when vitamin K antagonists (e.g., warfarin) or antibiotics are used.
2. Cautions regarding samples
(1)Use plasma or serum as a sample.
(2)Do not use preserved samples that may have been putrefied or degenerated.
(3)Thoroughly mix the sample before test. When frozen samples are thawed, the components may not be uniformly distributed.
(4)Samples may have been contaminated by hepatitis virus. Take precautions to avoid parental(e.g., via wounds) or oral infection.
3. Procedural precautions
(1)Testing should begin only after familiarity with the entire procedure is acquired.
In particular, it should be ensured that the cup is brought into intimate contact with the cup holder and that the reaction temperature and time are carefully controlled.
(2)The precision of pipettes and other apparatus plays an important role in the precision of the test. Abundant caution should be observed when selecting and operating pipettes and other apparatus. It is the users responsibility to validate pipettes and other apparatus for its intended use.
(3)The standard curve should be constructed with each test using the average of measurements made in duplicate.
(4)It is recommended that pooled sera or plasma with known concentrations of PIVKA-Ⅱ be included with each set of determinations to monitor results.
(5)The reagents should be prepared before using as a rule. When one kit is used at one time, the same reagent should be placed in one container. The same precaution should be observed when more than one kit of the same lot number is used at one time.

(6)The antibody coated cup should be used immediately after opened. Exercise care not to rub the insides of the cup with a pipette.
(7)When the same antibody coated cup is to be used several times, the aluminum foil packet should be tightly zippered after each use.
(8)When add enzyme-labeled antibody to the cups, care should be exercised to avoid soiling the periphery of the cup.
(9)Care should be exercised to avoid eye or hand contact with the substrate solution or the stop-reaction solution.
4. Disposition of waste materials and equipment used
All samples, reagents, and equipment used in the test should be disposed of by any of the following methods.
(1) Immersion in formalin solution(1 in 2,000) at 37℃ for 72 hr or more.
(2) Immersion in 2% glutaraldehyde solution for 1 hr or more.
(3) Immersion in a 1:50-60 dilution of hypochloride(12% sodium hypochloride) for 1 hr or more.
(4) If any of the above methods cannot be employed, autoclave at 121℃ for at least 1 hr.

RECOMMENDED STORAGE AND HANDLING OF KITS AND WASTE MATERIALS

1. Precautions
Reagents should be prepared when use, and care should be exercised in their storage.
Use the substrate solution immediately after prepared.
2. Storage
Refrigerate between 2-10℃(Do Not Freeze).
3. Effective period
The kit will be stable at 1 year from manufactured if recommended storage temperature is maintained. Do not use the kit beyond the expiry date stated on vial label and outer package.
4. Additional information
(1) The reagents contain sodium azide as a preservative, shown below. On disposal, flush with large volume of water.
Standard antigen (dissolved soln.), sample diluent and reaction solution: 0.1 w/v%
Stop-reaction solution: 0.01 w/v%
(2) Do not use the leftover wash solution for later use. Treatment of wastes generated by this test should be done according to guidelines in accordance with the local laws and regulations.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com