Sceti Medical Labo K.K.

Pancreatic Phospholipase A2 Kit

SML Pancreatic PLA2

Instruction Manual

General Cautions

1. For research use only.
2. Use only according to the use method described in this leaflet. Reliability of measurement values is guaranteed only when used according to the use method and purpose described in this leaflet.
3. This kit is a radiopharmaceutical agent; use is restricted to RI-controlled areas.
4. Diagnostic judgment by the attending practitioner should be based on the measurement results, along with the clinical symptoms and other related test results.

Purpose of Use

Measurement of volume of Pancreatic phospholipase A2 (pancreatic PLA2) in serum and plasma

Measurement Principles

This kit uses the solid phased single antibody method of radioimmunoassay.

In other words, the standard pancreatic PLA2 solution or the pancreatic PLA2 of an unknown specimen, and the pancreatic PLA2 indicated by Iodine 125, competitively react with the antibodies that are solid-phased to polyacrylamide microbeads. After reaction, they are separated by centrifuge, the supernatant is removed by aspiration, and the radiation of the precipitation is counted. The volume of the indicator pancreatic PLA2 is reduced according to the increase in the volume of the standard PLA2 solution or the pancreatic PLA2 of the unknown specimen.

The concentration of pancreatic PLA2 of the unknown specimen can be calculated from the standard curve that was prepared using the standard pancreatic PLA2 solution.

Reagents

One kit contains 10 kinds of reagents as described below, and can perform 50 measurements (double measurement for standard curve and 17 specimens).

*Iodized pancreatic PLA2 (125 I) test reagent･･･1 vial (freeze dried)

In 1 vial Iodized human pancreatic phospholipase A2

(pancreatic PLA2) (125I) on the test date 42.5kBq

*Solid-phase antibody test reagent ･･･1 vial (freeze dried)

In 1 vial mouse anti-human pancreatic phospholipase A2

(Pancreatic PLA2) Monoclonal antibody-binding microbeads Human pancreatic phospholipase A2

(Pancreatic PLA2) Volume to bind 5.17ng

*Pancreatic PLA2 Standards･･･1 vial each(freeze dried)（O, A～E）

Standards are provided at the following PLA2 Concentrations after preparation: 0,100,300,1000,3000 & 10000 ng/dL

*Precipitation stabilizer ･･･1 bottle (30mL)

The phosphate buffer fluid contains insoluble micro particles.

*Controlled serum test reagent ･･･1 vial (freeze dried)

To confirm the reliability of measurement, measure with the purpose of quality control in daily examination.

Caution during processing

1. Regarding the measurement specimen)

(1) Use serum or plasma

(2) When using plasma, either heparin, sodium citrate, EDTA-2Na, sodium fluoride, or oxalic acid may be used as the anticoagulant.

(3) When using a freeze dried specimen, it should be dissolved, returned to room temperature and mixed well but not foaming, prior to measurement.

2. Influential material1)

(1) Hemolysis

No effect was observed when hemoglobin (0-440mg/dL) was added to the serum specimen.

(2) Bilirubin

No effect was observed when bilirubin (0-21.9mg/dL) was added to the serum specimen.

(3) Chylous specimen

No effect was observed when chyle (formazinturbidity 0-2340 degree) was added to the serum specimen.

Dosage and Administration (Assay Procedures)

1. Preparation of the test reagent

(1) Iodinated pancreatic PLA2 (125I) solution (yellow)

Add 5.5mL of purified water to the iodinated pancreatic PLA2 (^{125}I) test reagent, and dissolve it.

(2) Antibody suspension (blue)

Add 11mL of purified water to the solid-phased antibody test reagent.

(3) Standard pancreatic PLA2 solution O

Add 5mL of purified water to the standard pancreatic PLA2 test reagent O, and dissolve it.

(4) Standard pancreatic PLA2 solution A, B, C, D, E

Add 500 μL of purified water to each standard pancreatic PLA2 test reagent A, B, C, D, E, and dissolve them. Concentrations after preparation of each reagent shall be 100, 300, 1000, 3000, and 10000 ng/dL.

(5) Precipitation stabilizer

Already prepared, use as is.

(6) Controlled serum test reagent

Add 500 μL of purified water to the controlled serum test reagent and dissolve it.

2. Preparation of the test reagent and handling cautions

(1) Gently dissolve the test reagent to avoid any foam.

(2) Pipette operation should be performed as accurately as possible.

(3) After adding 11mL of purified water to the vial, the solid-phased antibody test reagent should be thoroughly mixed and used in a uniformly suspended condition.

(4) The precipitation stabilizer has already been prepared, but should be used in the well-mixed and uniformly suspended condition.

3. Necessary devices and equipment

(1) Necessary devices

Transfer pipette (5mL)
Micro pipette (50 μL, 100 μL, 200 μL, 500 μL)
Measuring pipette (10mL)
Test tubes and tube rack
Aspirator

(2) Necessary equipment

Constant-temperature bath, centrifugal separator, vortex mixer, γ-counter

4. Measuring Procedures

Measurement should be performed in duplicate.

(1) For each concentration, insert 50 μL of each the standard pancreatic PLA2 solution, the control serum and the specimen, in a test tube.

(2) Add 100 μL of iodinated pancreatic PLA2 (125 I) solution to each test tube.

(3) Separately, as an additional total radiation measurement, insert 100 μL of iodinated pancreatic PLA2 (125 I) solution in each test tube, sealing immediately, and set aside until radiation measurement.

(4) Add 200 μL of antibody suspension to each test tube, and mix thoroughly.

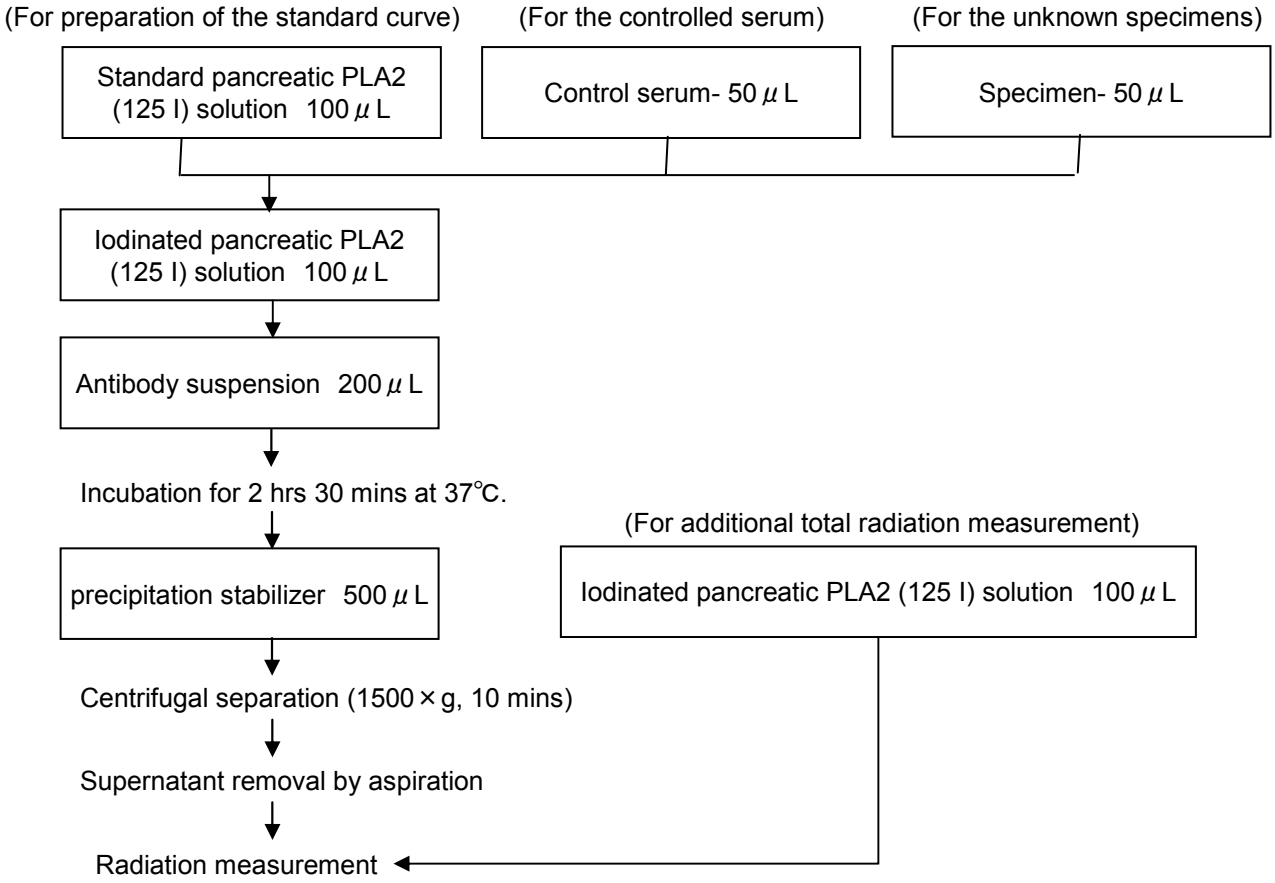
(5) Incubate for 2 hrs 30 mins at 37℃.

(6) After incubation, add 500 μL of precipitation stabilizer to each test tube, and mix thoroughly.

(7) Perform centrifugal separation on 1500 × g of each test tube for 10 mins, remove the supernatant by aspiration.

(8) Measure the radiation of each test tube by γ –counter, and calculate the count number of each.

5. Outline of the measurement procedures



6. Calculation of the results

Measurement examples: The following results were gained by γ-counter.

Test Tube No.	Sample description	Count number (cpm)	Count number (ave)	B/B0 (%)
① ②	Additional total radiation measurement	37633 37768	(T) 37700.5	
③ ④	Standard pancreatic PLA2 O (0ng/dL)	14933 14881	(B0) 14907	
⑤ ⑥	A (100ng/dl)	13136 13108		88.12 87.93
⑦ ⑧	B (300ng/dL)	10236 10190		68.67 68.36
⑨ ⑩	C (1000ng.dL)	5794 5823		38.87 39.06
⑪ ⑫	D (3000ng/dL)	2858 2859	(B)	19.17 19.18
⑬ ⑭	E (10000ng/dL)	1341 1334		9.00 8.95
⑮ ⑯	Controlled serum	6980 6960		46.82 46.69
⑰ ⑱	Unknown specimen	6750 6777		45.28 45.46

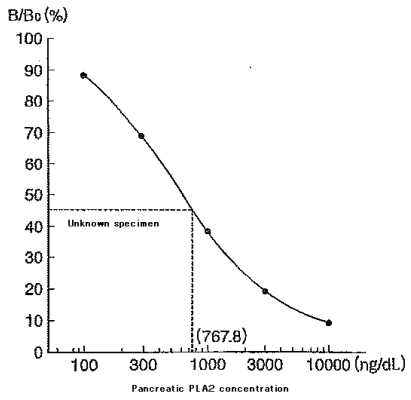
(1)B/BO(%) of each standard pancreatic PLA2 solution is calculated by the following formula.
B/B0(%) = Count number of each standard pancreatic PLA2 solution / Average count number of standard pancreatic PLA2 solution 0(B0）x 100

(2)By using a single logarithmic graph, each concentration of each standard pancreatic PLA2 solution is given as the horizontal axis (logarithmic scale), and B/Bo(%) as the vertical axis, the standard curve is created by plotting each B/B0(%) of each standard pancreatic PLA2 solution gained by (1).

(3)For the specimen and controlled serum, B/B0(%) is calculated by the following formula in the same manner.
B/B0(%) = Count number of specimen or count number of the controlled serum / Average of count number of standard pancreatic PLA2 solution 0(B0) x 100

(4)By using the standard curve, the pancreatic PLA2 concentration (ng/dL) is gained from B/B0(%) of the specimen and controlled serum.

7. A case of the standard curve



Judgment of measurement results

Standard range

While setting the standard range at each facility is recommended, the following values can be used for convenience.

Standard range: 130-400ng/dL

Clinical significance

Pancreatic PLA2 sensitively reflects a reduction in pancreatic exocrine function, and is useful in the diagnosis of acute pancreatitis, rapid deterioration of chronic pancreatitis, pancreatic cancer, and in follow-ups.3)-12)

Performance

1. Sensitivity test

(1) Standard curve

When testing each standard pancreatic PLA2solution, the intercept point of the logit (B/B0(%) and log X regression line was 5.0-8.0, with a slope of -2.8~-1.8.

(2) Binding rate

Binding rate: 25% or more

Binding rate B0/T(%) = Average count number of the standard pancreatic PLA2 solution O / Average count number of the sample for additional total radiation measurement (T) × 100

2. Accuracy

When measuring a specimen for the control of a known concentration, accuracy is within ±20% of the known concentration.

Furthermore, regarding 3 lots of this kit, when measuring the serums L, M, H for control three times, the following results were gained.

		Serum for control		
		L (indicated value) 289ng/dL	M (indicated value) 797ng/dL	H (indicated value) 3560ng/dL
Range	(ng/dL)	262～311	755 ～841	3368 ～3971
	(%)	90.7 ～107.6	94.7 ～105.5	94.6 ～110.3

3. Repeatability test

When the same specimen was measured five times simultaneously, the C.V. value of the measurement value was less than 10%.

Furthermore, when the serums L, M, H for control were measured five times simultaneously, the following results were gained.

		Serum for control		
		L (indicated value) 289ng/dL	M (indicated value) 797ng/dL	H (indicated value) 3560ng/dL
Range	C.V value (%)	1.6～6.4	0.7 ～4.4	1.2 ～6.4

4. Measurement Range
   100-10000ng/dL
5. Standard material for calibration
   (1) Separation and purification method of human pancreatic phospholipase A2 (pancreatic PLA2) standard material
   After dialyzing the human pancreatic fluid, centrifugal separation was performed and then supernatant purification by hydrophobic chromatography and ion-exchange chromatography, and then freeze dried. The matter dissolved by the appropriate amount of solvent was determined to be pancreatic PLA2 reference material.
   (2) The determinate quantity of the human pancreatic phospholipase A2 (pancreatic PLA2) standard material
   Regarding pancreatic PLA2 standard material, the determinate quantity was set by amino acid analysis, with phenylalanine as the standard.

Caution during Use and Handling

1. Caution while handling (hazard prevention)
   (1) Do not ingest or taste the test reagents.
   (2) Avoid contact of the test reagents with eyes and skin.
   (3) In the event the test reagent comes in contact with the mouth, eyes or skin, take immediate emergency measures, such as washing thoroughly and flushing with water. If ill, consult a doctor.
   (4) When handling a specimen, exercise caution and wear disposable gloves to avoid infection.
   (5) The control serum test reagent includes serum that contains human-derived ingredients which have been tested negative for HIV antibodies, HBs antigens, and HCV antibodies, but caution should still be exercised in the same manner as a specimen.
2. Cautions during use
   (1) Upon delivery, this kit should be stored under refrigeration (2-8℃) according to the storage directions. When each constructive test reagent that is stored under refrigeration (2-8℃) for measurement is to be used, it should be returned to measurement room temperature (20-30℃) prior to use.
   (2) Do not use expired test reagents, as the reliability of the measurement value cannot be guaranteed.
   (3) The test reagents are prepared to provide accurate reaction by the combination of test reagents with the same serial number within the kit. Do not combine or mix test reagents with different serial numbers from within the kit.
   (4) The bottles of freeze dried test reagents are under negative pressure. Use care when opening to avoid splashing/spilling the test reagents.
      Corresponding test reagent ingredients:
      Iodinated pancreatic PLA2 (125 I) test reagent
      Solid-phased antibody test reagent
      Standard pancreatic PLA2 test reagent (O, A,B,C, D, E)
      Controlled serum test reagent
   (5) A standard curve should be prepared for each measurement.
   (6) Measurement results are influenced by reaction conditions (incubation time, temperature, etc.). The standard pancreatic PLA2 solution and specimen should be processed under the same designated conditions simultaneously.
   (7) To avoid risk of contamination by the test reagents, which contain a radioactive ingredient, do not use the containers in this kit for any other purpose.

3. General cautions regarding radiopharmaceutical agents for in vitro diagnosis

(1) This kit is a designated drug.

(2) Since iodinated pancreatic PLA2 (125 I) test reagent, which is included as the test reagent ingredient of this kit, contains Iodine 125, a designated radioactive compound, it should be stored in a specified place, and strictly handled in accordance with the rules of use for the handling of radioactive ingredients, in addition to the following general cautions.

1) To avoid contamination of the body, wear disposable gloves, professional protection clothing and protective gear in the working room.

2) To avoid accidentally ingesting the radioactive ingredients during work, do not perform pipette operation by mouth. Also, do not eat, drink, smoke, and wear make-up in the working room.

3) Dispose of radioactive effluent or radioactive waste according to the prescribed regulations. Also, do not hinder other RI tests. Furthermore, consider ways to avoid unnecessary radiation.

4) If a radioactive ingredient is accidentally spilled, completely wipe off with burnable material immediately, and dispose of as radioactive waste. Carry out a contamination inspection after disposal.

4. Cautions regarding disposal

(1)The following test reagent ingredients contain approximately 0.1w/v% of sodium azide as a preservative. When disposing of solution after use, etc., be sure to thoroughly dilute the solution by slowly flushing large amounts of water, to avoid the explosive metal azide material from reacting with the metal in the drain pipes, such as lead or copper.

Corresponding test reagents ingredients:
Iodinated pancreatic PLA2 (125 I) test reagent
Solid-phased antibody test reagent
Standard pancreatic PLA2 test reagent (O, A,B,C, D, E)
Precipitation stabilizer

(2) Any utensils, test reagents, or containers of the test reagent which have come in contact with the specimen should be deemed as contaminated, and should be sterilized by autoclave, or by soaking in an antiseptic solution, such as 1% hypochlorous acid.

(3) Test reagents, utensils, etc, should be disposed of in accordance with the laws regarding waste disposal and regulations such as the Water Pollution Control Law.

Storage / Expiration Date

1. Storage	Store at 2-8℃ (under refrigeration) in a dark place.
2. Expiration date	9 weeks from the assay date (the expiration date is displayed on each individual box, etc.)

Package Unit

1 kit contains 50 measurements

Iodinated pancreatic PLA2 (125 I) test reagent・・・1 vial
Solid-phased antibody test reagent ・・・1 vial
Standard pancreatic PLA2 test reagent (O, A, B, C, D, E) ・・・1 vial each
Precipitation stabilizer ・・・1 bottle
Control serum test reagent ・・・1 vial

Main References

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