For research use only

Reagent for determination of human pancreatic lipase in serum and plasma

LIPASE KIT S

■Introduction and Characteristics

Pancreatic lipase is secreted from the pancreatic acinar cells and excreted into the duodenum through the pancreatic duct. The enzyme is leaked into the circulation in case of obstruction or stricture of the pancreatic duct, or destruction of the pancreatic tissues. Therefore, the serum and plasma lipase of pancreatic origin is considered to be a useful index for the diagnosis of pancreatic diseases.

LIPASE KIT S is a kit for determining the enzyme activity of pancreatic lipase in serum and plasma based on the combination of S-acyl artificial substrate, BALB*, and DTNB, reagent for SH assay. This kit has the following characteristics.

1. LIPASE KIT S can determine the activity of pancreatic lipase with good reproducibility by using 50 µL of serum and plasma precisely.
2. LIPASE KIT S does not require any laborious processes such as extraction and titration.
3. LIPASE KIT S can carry out a multi-sample assay in a short time (ex. 50 samples within 1 hour).
4. LIPASE KIT S can be performed by using ordinary experimental tools. It does not need any special apparatus.

*2,3-dimercapto-1-propanol tributyrate

■Contents of LIPASE KIT S

Each kit (for 100 assays) contains the following reagents.

Substrate solution		1 bottle (22 mL)
	2,3-dimercapto-1-propanol tributyrate (BALB)	6.69 mg/mL
	Sodium dodecyl sulfate (SDS)	5.73 mg/mL
Esterase inhibitor solution		1 bottle (4.4 mL)
	Phenylmethylsulfonyl fluoride (PMSF)	3.48 mg/mL
Chromogenic reagent		1 bottle (240 mg)
	5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB)	0.1 mg/mL
Buffer solution		1 bottle (25 mL)
Reaction terminator concentrate		1 bottle (25 mL)

■Application

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Determination of pancreatic lipase in human serum and plasma

■Principle

1. The pancreatic lipase in serum and plasma hydrolyzes BALB to give BAL* and butyric acid in the presence of SDS and PMSF. In this process, SDS works as an activator of lipase, and PMSF in combination with SDS works as an inactivator of esterase except for lipase in human samples.
2. The liberated BAL reacts with DTNB, a reagent for SH assay, to release yellow TNB anion quantitatively.
3. The lipase activity can be measured by the colorimetry of TNB anion after stopping the reaction and clarifying the turbid reaction mixture by the addition of reaction terminator.

   * BAL: British anti-Lewisite, Dimercaprol, 2,3-dimercapto-1-propanol

■Assay procedure

1. Instruments and materials required

Pipettes with disposable tips 20, 50, 100, 1000 µL, Whole pipette, Volumetric cylinder 500 mL, Erlenmyer flask 500 mL, Glass test tube (12 mmφ x 100 mm), Tube stand, Incubator (30±1°C), Dispenser (2 mL), Photometer for measuring absorbance at 410-420 nm, Stop-watch, Shield board

2. Preparation of sample

Use serum or plasma as a sample

3. Preparation of reagents

(1) Stock solution of Chromogenic buffer

Add 2.4 mL of Buffer solution to Chromogenic reagent in a bottle and dissolve completely, and then add 22 mL of purified water. Although the resultant solution can be used several times within 3 months when kept frozen, preferably, use the solution with absorbance of less than 1 at 412 nm.

(2) Working solution of Chromogenic buffer

Mix one volume of the Stock solution of Chromogenic buffer with one volume of Buffer solution, and then add 8 volume of purified water. (As the Working solution of Chromogenic buffer is unstable around room temperature, keep it under icd-chilled condition at use.)

Examples for preparing the working solutions of Chromogenic buffer

	For 10 assays	For 20 assays	For 50 assays
Stock solution of Chromogenic buffer	2.2 mL	4.4 mL	11 mL
Buffer solution	2.2 mL	4.4 mL	11 mL
Purified water	17.6 mL	35.2 mL	88 mL
Prepared volume	22.0 mL	44.0 mL	110 mL
Necessary volume	20.0 mL	40.0 mL	100 mL

(3) Reaction terminator

As Reaction terminator concentrate is solid at low temperature, thaw it by warming at 30°C for 5-10 min. Pour the whole volume of the Reaction terminator concentrate into a 500-mL volumetric cylinder, and then adjust the volume to 500 mL. After the preparation of the Reaction terminator, it can be kept at 2-8°C for 3 months. When a crystalline mass is observed during the storage at low temperature, use it after dissolving completely.

(4) Substrate solution, Esterase inhibitor and Buffer

Use as they are without any treatment.

4. Procedure

(1) In the assay for one sample, provide for two glass tubes A and B, which can be used for centrifugation. Use A for the test sample and B for the blank sample.

(2) Into A and B test tubes, pipette each 1 mL of Chromogenic working solution and each 50 µL of human serum or plasma samples. Then, add 20 µL of the Esterase inhibitor solution to each test tube.

(3) Mix them well and incubate at 30±1°C for 5 min. [Esterase inhibition reaction]

(4) Add 100 µL of Substrate solution only to test tube A and mix well, and immediately incubate test tubes A and B at 30±1°C for 30 min. [Enzyme reaction]

(5) Stop the reaction by adding 2 mL of Reaction terminator at the same interval of the addition of the Substrate solution to both test tubes and agitating vigorously. Add 2 mL of the Reaction terminator to test tube B and mix well.

(6) Add 100 µL of Substrate solution only to test tube B, then mix well. After the mixing, they must be kept shielded from light.

(7) Measure the absorbance of the test sample (A) and the blank sample (B) at 412 nm (light length 1 cm) against purified water as a reference. (The developed color is unstable against any light and it is decolored in a long period. Therefore, the

absorbance should be measured within 1 hour after the enzyme reaction is terminated.) [Measurement of absorbance]

5. Calculation of lipase activity

(1) One thousand times of the absorbance differences between those of the test sample (A) and the blank sample (B), namely absorbance (A−B) x 1,000 is defined as BALB lipase unit/0.05 mL/30 min. BALB units can be converted into international units (IU/L) by multiplying by a factor of 0.147 (IU/L; μmol of liberated SH groups of BAL/min/L of serum or plasma at 30°C).

Example

Serum sample	A	B	A−B	BALB Unit	IU/L
A healthy subject	0.152	0.070	0.082	82	12
A patient with reoccurring chronic pancreatitis	0.908	0.095	0.813	813	120

(2) When a value less than the lower detection limit (50 BALB Unit) is obtained, it should be reported as "<50 BALB Unit".

(3) When a value more than 1,000 BALB Unit is obtained, the incubation time of enzyme reaction for the sample is shorten to 15 min (or 10 min) to make the absorbance adjust less than 1 and multiply the date obtained via the procedure (5)-(7) by 2 times (or 3 times).

■Cautions upon assay processes

1. Samples

Use human serum or plasma as a sample for this kit.

2. Preparation of sample

When a plasma sample is collected, heparin, citrate and oxalate can be used as an anticoagulant.

3. Interfering substances

Hemoglobin up to 200 mg/dL and bilirubin up to 10 mg/dL are the maximum permissible level of interfering substances.

4. Freezing-thawing of samples

No influence on the determination is observed after 10 cycles of freezing-thawing.

5. Storage of samples

Store tubes containing samples at 2-8°C. In the case of storage for a long period,

sample tubes should be kept frozen. (In the frozen state, lipase activity can be kept in 1 year.) When frozen samples are used for the assay, thaw the samples at room temperature gradually and mix well before use.

■Judgment of the result of determination

(Normal range of lipase activity)

Average lipase activities of serum samples from healthy subjects at three different institutes are 70-75 BALB units as shown below in the Table. The frequency of lipase activity at different age indicates a normal distribution of which peak is around middle age. The upper reference value of normal subject is 150 BALB units (average + 2SD).

Lipase activities of serum samples from normal subjects

Reporters	Lipase activity (BALB units) Mean ± SD	Number of subjects	Age range of subjects (years old)
Kurooka, et al.[3]	74 ± 37	200	10-45
Morishita, et al.[6]	75 ± 34	159	2-65
Furukawa, et al.[9]	70 ± 22	112	1-59

■Performance

1. Sensitivity

(1) The absorbance difference between the test sample and the blank sample is less than 0.05 when purified water is used as a test sample.

(2) The absorbance difference between the test sample and the blank sample is less than 0.15 when three normal serum samples are pooled and used as a test sample.

2. Specificity

Control serum (lipase activity, 400-600 BALB units) should show a value within 90-110% of its known activity with this kit.

3. Reproducibility

When two distinct samples (lipase activity, 200-300 and 400-600 BALB units) are determined 10 times each simultaneously, the coefficient of variation in the absorbance difference between the test sample and the blank sample should be less than 5%.

4. Range of measurement

Lipase activity 50-1,000 BALB units

■Correlation between this kit and other methods

Correlation between LIPASE KIT S and other methods

Other methods	This kit	n	r	Regression	Reporter
RI assay (Y)	(X)	16	0.979	Y=17.13X + 386	Furukawa, et al.[9]
Alkaline titration (X)	(Y)	83	0.917	Y=152X − 94	Kuchi, et al.[10]
Alkaline titration (X)	(Y)	89	0.768	Y=1.3X − 38	Rick, et al.[12]
Turbidimetry (X)	(Y)	77	0.989	Y=233X − 124	Kansai Medical School
Turbidimetry (Y)	(X)	51	0.781	Y=0.62 + 69	Matsumoto, et al.[13]

■Precautions for use or handling

1. Precautions for use

(1) General precautions

1) Do not use the kit for any purpose not mentioned in this manual.
2) Do not use reagents after their expiration date.
3) Be sure to use reagents, including Buffer and Reaction terminator concentrate, of the same lot. Do not use in combination with any reagents from different lots.
4) Do not use the reagents if all reagents included in the kit before used and/or all the reagents except for Stock solution of Chromogenic buffer are frozen mistakenly.
5) If the reagents of this kit get into the eyes or mouth, applying appropriate first aid such as thorough washing, and then consult a doctor if necessary.
6) Before using the kit, be sure to read the operating manuals for the equipment required for this assay.

(2) Precautions for procedure

1) All reagents should be added in the exact order stated in the procedures. Assay the test sample and the blank sample in the same condition.
2) Shield the assay tubes from light during the incubation time and the period by the time for measuring absorbance after the reaction termination. Measure the absorbance within 1 hour after the reaction termination. For shielding the incubator and test tubes from light, covering board with dark color or box will do.
3) Avoid the contamination of sample and reagents with microorganisms. Avoid the cross-contamination between reagents.

2. Avoiding hazards to the user

(1) Viruses

As any human serum and animal serum is not used in this kit, there is no possibility of infection with HBV, HIV and HCV. However, treat the reagents as if they were infectious, such as clinical samples containing viruses. Inactivate any viruses in samples and used apparatuses when the test is completed by the following methods.

1) Submerge in 3% sodium dodecyl sulfate solution at 100°C for 5 min.
2) Autoclave at 132°C for 1 hour.
3) Submerge in 1 mol/L sodium hydroxide solution at room temperature for 1 hour.
4) Submerge in 1-5% sodium hypochlorite solution at room temperature for 2 hours.

(2) Pipetting

Never use your mouth to pipette the reagents or samples at any time. Never fail to use a pipette with disposable tips.

3. Handling of waste

Reagents in this kit contain sodium azide, which is reactive to lead and copper, and sometimes explosive metal azide as a resulting compound is generated. When the reagents are discarded, wash them with much volume of water. Inactivate the test samples and the blank samples according to the session 2.(1) Viruses, and then wash them with much water.

4. Precautions for diagnosis

Clinical diagnosis should be done on the basis of lipase activity by this kit in combination with other clinical aspects by medical doctors.

■Storage method and expiry period

Storage: Store in a cool place (2-8°C), protected from light.
Expiry period: 3 years

■Package units

LIPASE KIT S: 1 kit (100 tests)

■References

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