LIPASE-PS™

REF 805 1 Kit

Pour d'autres langues | Para outras línguas
Für andere Sprachen | Για τις άλλες λώσσες
Para otras lenguas | För andra språk
Per le altre lingue | For andre språk
Dla innych języków | For andre sprog

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INTENDED USE

Lipase-PS™ Reagent is intended for the quantitative, kinetic determination of pancreatic lipase (triacylglycerol acylhydrolase, E.C. 3.1.1.3) activity in serum at 550 nm.

SUMMARY

The measurement of serum lipase activity is widely used for the diagnosis of acute pancreatitis. A 10-fold increase of lipase activity above the upper reference limit is suggestive of pancreatitis, pancreatic injury, or inflammation of organs contiguous to the pancreas.[1] It is recommended that other tests, such as trypsinogen and amylase isoenzymes be performed to supplement the diagnosis.[2]

Pancreatic lipase hydrolyzes the α-1 and α-3 esters of long chain fatty acids from their triglycerides. The enzymic activity requires the presence of co-lipase which facilitates the binding of substrate to the enzyme. The Cherry-Crandall titrimetric method[3] is based on the titration of fatty acids, whereas in the turbidi-metric method of Shihabi and Bishop[4] pancreatic lipase activity is monitored by the decrease of turbidity in the triglyceride emulsion. Other approaches to lipase assay include colorimetric, nephelometric, and fluorometric methods.[5,6,7]

The Trinity Biotech procedure is based on the colorimetric method of Imamura, et al.[8] 1,2-Diglyceride is hydrolyzed to 2-monoglyceride and fatty acid. 2-Monoglyceride is then measured by coupled enzyme reactions catalyzed by monoglyceride lipase, glycerol kinase, glycerolphosphate oxidase, and peroxidase. The test is highly sensitive and specific for pancreatic lipase using co-lipase and deoxycholate as activators. The assay is simple to perform and can easily be adapted to automated analyses.

PRINCIPLE

The enzymatic reactions involved in the pancreatic lipase assay are as follows:

1,2-diglyceride	pancreatic lipase →	2-monoglyceride + fatty acid
2-monoglyceride	MGLP →	glycerol + fatty acid
glycerol + ATP	GK →	glycerol-3-phosphate + ADP
glycerol-3-phosphate + O_2	GPO →	DAP + H_2O_2
H_2O_2 + 4-AAP + TOOS	POD →	quinone diimine dye + $4H_2O$

Serum pancreatic lipase catalyzes the hydrolysis of a natural 1,2-diglyceride to form monoglyceride and fatty acid. Monoglyceride is hydrolyzed by mono-glyceride lipase (MGLP) to form glycerol and fatty acid. Glycerol is then phosphorylated by glycerol kinase (GK) in the presence of ATP to form glycerol-3-phosphate which is oxidized by glycerol-3-phosphate oxidase (GPO) to form dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). Subsequently, H_2O_2 reacts with 4-aminoantipyrine (4-AAP) and sodium N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (TOOS) in the presence of peroxidase (POD) to form a quinone diimine dye. The dye absorbs light at 550 nm. The rate of increase in absorbance at 550 nm is directly proportional to the pancreatic lipase activity in the sample.

REAGENTS

The Trinity Biotech Lipase-PS Reagents, when reconstituted according to the instructions, have active ingredients with approximate concentrations as follows:

LIPASE-PS SUBSTRATE REAGENT

1,2-Diglyceride	1.1 mmol/L
TOOS	2 mmol/L
ATP	0.66 mmol/L
Monoglyceride lipase (microbial)	860 U/L
Glycerol kinase (microbial)	1340 U/L
Glycerol-3-phosphate oxidase (microbial)	40,000 U/L
Peroxidase (horseradish)	1340 U/L
Co-lipase (porcine)	40,000 U/L
Buffer and nonreactive stabilizers	

LIPASE-PS SUBSTRATE DILUENT

Cholic acid	5.34 mmol/L
Sodium azide	0.05%
Buffer	

LIPASE-PS ACTIVATOR REAGENT

Deoxycholate	36 mmol/L
4-Aminoantipyrine	6 mmol/L
Sodium azide	0.05%
Buffer	

LIPASE-PS STANDARD

Pancreatic lipase (human)	Activity on label
Sodium azide	0.05%
Buffer and nonreactive stabilizers	

No international reference standard is available. Calibrator values were assigned using internal reference material.

PRECAUTIONS

Do not substitute lipase substrate, lipase diluent, lipase activator or lipase standard from kits with different lot number or from other manufacturers.

Lipase-PS reagents are for "In Vitro Diagnostic Use". Normal precaution exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.

CAUTION

Avoid contact and inhalation of Lipase-PS Substrate. Avoid pipeting reagent by mouth. Take precautions to avoid microbial contamination.

Lipase-PS Substrate Diluent, Standard and Activator Reagent contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. Avoid azide accumulation.

Lipase-PS Standard is a POTENTIALLY BIOHAZARDOUS MATERIAL. Source materials from which this product was derived were found negative for HBsAg and for antibodies against HCV, HIV-1 and HIV-2 by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, this product should be handled observing the same safety precautions employed when handling any potentially infectious material.

When Lipase-PS is used on automated analyzers after either triglyceride or cholesterol tests, ensure that all tubing and probes are well cleaned to avoid contamination with lipase, cholesterol esterase, or cholesterol oxidase from previous assays.

Refer to Material Safety Data Sheets for any updated risk, hazard or safety information.

PREPARATION

Lipase-PS Substrate Diluent and Activator Reagent are supplied ready for use. Reconstitute Lipase-PS Substrate Reagent with volume of Substrate Diluent indicated on vial label. Mix by inversion. DO NOT SHAKE.

Lipase-PS Standard solution is prepared by adding 3.0 ml deionized water to vial. Mix by gentle swirling until material is completely dissolved.

STORAGE AND STABILITY

Store reagents in refrigerator (2–8 °C). Reagents are stable until expiration date shown on labels.

Substrate Reagent solution is stable for 24 hours at room temperature (18–26 °C) or 28 days refrigerated (2–8 °C) when protected from light. Lipase-PS Standard solution is stable for 30 days refrigerated (2–8 °C) when protected from light.

DETERIORATION

Lipase-PS reagents are not suitable for use if microbial growth is evident or if the absorbance of substrate reagent solution measured at 550 nm vs water as reference is greater than 0.200.

AUTOMATED INSTRUMENTS

Application procedures using Trinity Biotech Lipase-PS Reagent are available for various automated instruments. Please contact Trinity Biotech Technical Services Department (USA: 1-800-325-3424; Ireland: +353 1 2769800) for more information.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Serum is the specimen of choice. Collect blood carefully to avoid hemolysis and separate serum promptly afterward. Lipase activity in serum is stable for several weeks when stored refrigerated at 2–8 °C or frozen until use. Lipase can be inactivated by repeated freezing and thawing.

INTERFERING SUBSTANCES

Microbial lipase and cholesterol esterase affect the assay. Young et al.,[9] have listed certain drugs and other substances which are known to interfere with lipase activity.

MANUAL PROCEDURE

MATERIAL PROVIDED

Lipase-PS Substrate Reagent	Lipase-PS Activator Reagent
Lipase PS Substrate Diluent	Lipase-PS Standard

MATERIALS REQUIRED BUT NOT PROVIDED

- Spectrophotometer, capable of accurately measuring absorbance between 540 and 560 nm, with temperature controlled cuvette compartment
- Cuvettes with optical properties suitable for use between 540 and 560 nm
- Pipeting devices for accurate delivery of volumes required for the assay
- Timer

PROCEDURE

1. Set spectrophotometer wavelength between 540 and 560 nm (for example: 550 nm) and absorbance reading to zero with water as reference. Set temperature of cuvette compartment to 37 °C.
2. Set up a series of cuvettes for BLANK, STANDARD, and SAMPLES.
3. Pipet 0.9 ml (900 µl) Substrate solution into each cuvette.
4. Add 0.015 ml (15 µl) deionized water, Standard solution, or serum samples into cuvetttes labeled as BLANK, STANDARD, and SAMPLES, respectively.
5. Mix by gentle inversion and incubate for **3–5 minutes** at 37 °C.
6. Add 0.3 ml (300 µ) Activator Reagent and mix by gentle inversion. Incubate for **3 minutes** at 37 °C.
7. Read and record absorbance of all cuvettes at 540–560 nm (for example: 550 nm) versus water as a reference. This is the INITIAL A.
8. Continue incubation at 37 °C. Exactly 2 minutes after Initial A, read and record absorbance of all cuvettes. This is the FINAL A.
9. To determine lipase activity (U/L) in samples, refer to "Calculations" section.

NOTE: If spectrophotometer accommodates larger volumes, the volumes of reagent and samples may be proportionally increased.

CALIBRATION

A lipase standard included with each series of assays must be used to calculate lipase activity of samples. The procedure is linear up to a lipase activity of 750 U/L.

QUALITY CONTROL

A control range should be established by the laboratory to determine the allowable variation in day-to-day performance. Failure to meet Quality Control specifications should be investigated and resolved. Re-calibration is suggested whenever control materials are not within the established acceptable range. The assay should then be repeated.

CALCULATIONS

Calculate lipase activity as follows:

ΔA_{BLANK} = FINAL A_{BLANK} – INITIAL A_{BLANK}

$\Delta A_{STANDARD}$ = FINAL $A_{STANDARD}$ – INITIAL $A_{STANDARD}$

ΔA_{SAMPLE} = FINAL A_{SAMPLE} – INITIAL A_{SAMPLE}

$$\text{Lipase activity (U/L)} = \frac{\Delta A_{SAMPLE} - \Delta A_{BLANK}}{\Delta A_{STANDARD} - \Delta A_{BLANK}} \times \text{Lipase Activity (U/L) of Standard}$$

NOTE: Multiply the values determined at 25 °C and 30 °C by 1.82 and 1.37 respectively, if the results are to be expressed at 37 °C.

EXAMPLE

When a serum sample was assayed by the above procedure for lipase activity, the following absorbance values were obtained:

	Blank	Sample	Standard
INITIAL A	0.0609	0.1241	0.8890
FINAL A	0.0725	0.1940	0.9963
ΔA	0.0116	0.0699	0.1073

$$\text{Lipase Activity (U/L)} = \frac{0.0699 - 0.0116}{0.1073 - 0.0116} \times 250^{*} = 152 \text{ U/L}$$

* Activity of Lipase Standard

LIMITATIONS

Samples with pancreatic lipase activity higher than 750 U/L should be diluted with isotonic saline and re-assayed. Multiply the result by the dilution factor to compensate for dilution.

EXPECTED VALUES

The expected range of serum lipase activity as determined by this lipase assay procedure was found to be from 7–60 U/L (37 °C). It is recommended that each laboratory establish an expected range characteristic for the local population.

PERFORMANCE CHARACTERISTICS

COMPARISON

A group of 107 serum samples with lipase activities ranging from 6–218 U/L was assayed by the described procedure and a commercially available turbidimetric method. Comparison of results yielded a correlation coefficient of 0.92 and the regression equation was y = 0.38x + 5.98.

PRECISION

Within-run and run-to-run precision studies yielded the following data:

Within-Run

	Serum 1	Serum 2	Serum 3
Mean (U/L)	20.9	191.8	325.2
Standard Deviation (U/L)	1.5	4.9	7.9
Coefficient of Variation (%)	7.3	2.6	2.4
Number of Assays	20	20	20

Run-to-Run

	Serum 1	Serum 2	Serum 3
Mean (U/L)	24.3	194.8	329.7
Standard Deviation (U/L)	2.3	2.3	4.6
Coefficient of Variation (%)	9.6	1.2	1.4
Number of Assays	20	20	20

SENSITIVITY

An absorbance change of 0.0022 per minute at 550 nm corresponds to lipase activity of 10 U/L at 37 °C when a spectrophotometer typically found in clinical laboratories is used for the measurement under the stated conditions.

REFERENCES

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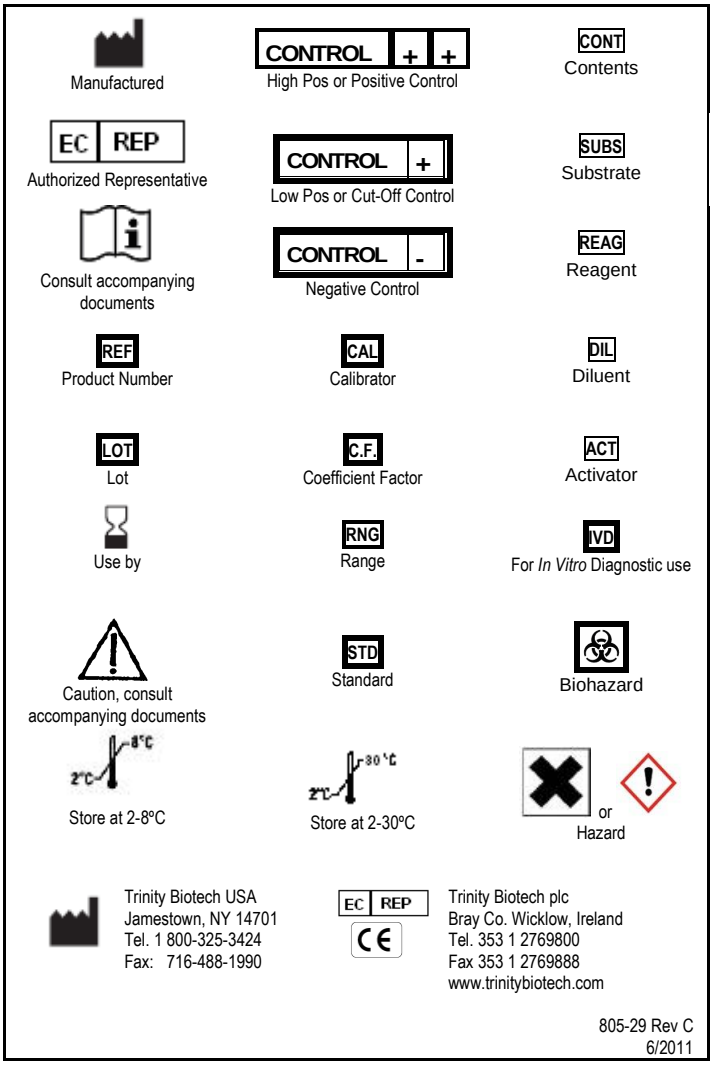
ORDERING INFORMATION

KIT		805-A Lipase-PS
Catalog No.	Item	Quantity
805-1A	Substrate Reagent	3 x 10 mL
805-2A	Substrate Diluent	30 mL
805-3A	Activator Reagent	10 mL
805-4A	Standard	3 mL

Reagents are available in kit only and cannot be ordered separately. Maximum assays per kit are instrument dependent.



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