LIPASE LR liquid reagent

REF 475LPS 5x10ml

IVD CE For in vitro medical device

Use

Kit for measurement of Lipase in serum.
Kinetic Colorimetric method

Summary

Lipase (LPS) is a pancreatic enzyme necessary for the absorption and digestion of nutrients that catalyzes the hydrolysis of glycerol esters of fatty acids. Determination of LPS is used for diagnosis of diseases of pancreas such as acute and chronic pancreatitis and obstruction of the pancreatic duct[1,7,8]. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle

The pancreatic lipase in presence of colipase, desoxycholate and calcium ions, hydrolyses the substrate 1-2-O-dilauryl-rac-glycero-3-glutaric acid-(6' -methylresorufin)-ester. The sequence of reactions involved in the enzymatic direct lipase determination is the following:

1-2-O-dilauryl-rac-glycero-3-glutaric-(6' -methylresorufin)-ester

$$\xrightarrow{\text{Lipase}}$$ 1-2-O-dilauryl-rac-glycerol + Glutaric-6'-methylresorufin-ester (no stable) $$\xrightarrow{OH^-}$$ Glutaric acid + Methylresorufin

The rate of methylresorufin formation, measured photometrically, is proportional to the catalytic concentration of lipase present in the sample.

REAGENTS

R 1a Buffer	TRIS pH 8.3 Colipase Desoxycholate Taurodesoxycholate	40 mmol/L ≥ 1 mg/L 1.8 mmol/L 7.2 mmol/L
R 1b Substrate (micro-emulsion)	Tartrate pH 4,0 Lipase Substrate Calcium chloride ($CaCl_2$)	15 mmol/L ≥ 0.7 mmol/L 0.1 mmol/L
LIPASE CAL	Standard. Lyophilised human serum The LPS activity (U/L methylresorufin at 37°C) is indicate on the label of the vial.	

Reagent Preparation

- **R1 – R2** Ready to use. Stability after opening 90 days at 2-8°C.
- **R2** Mix gently before use
- **LIPASE CAL**: Dissolve with 1 mL of distilled water. Cap and mix gently to dissolve contents. Stability: 7 days at 2-8°C or 3 months at –20°C; aliquote into small volumes and freeze.

Storage and stability

- Store the kit at 2-8°C.

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date

- After opening, the R1 vial is stable 90 days if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.

Precaution in Use

The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998). However the reagent should be handled with caution, according to good laboratory practice. Caution: the reagents contain Sodium Azide (0.095%) as preservative. Avoid swallowing and contact with skin, eyes and mucous membranes.
LIPASE CAL Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious

Waste Management

Please refer to the local legal requirements.

Specimen Collection and Preparation

- Serum or plasma with sodium citrate, EDTA or heparin
- Avoid repeated frozen and unfrozen
- Lipase is stable in the samples up to 2 days at 2-8°C.

Note

- The kit, according to this method, must be used in manual procedures. About automatic using follow specific applications.
- Avoid direct light, contamination and evaporation.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the single vials.

Procedure

Wavelength	λ: 580 nm
Working Temperature	37°C
Optical path	1 cm
Reaction	"kinetic"

Pipette into a cuvette:

	BLANK	STD	SAMPLE
Reagent R1	1000 µl	1000 µl	1000 µl
Reagent R2	200 µl	200 µl	200 µl
Distilled Water	10 µl	--	--
Sample	--	--	10 µl
Standard	--	10 µl	--

Mix, then incubate for 1' at 37°C Read initial absorbance (A) of the sample , start the stopwatch and read the absorbance at 1 minute intervals thereafter for 2 minutes.
Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

Calculations

(ΔA/min) Sample - (ΔA /min) Blank = (ΔA /min) of sample

(ΔA/min) Standard - (ΔA/min) Blank= (ΔA/min) of Standard

$$\frac{\Delta A/min\ \ Sample}{\Delta A/min\ \ Standard}$$ x Calibrador activity = U/L of lipase in the sample

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Quality controls

It's necessary, every time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert.Each laboratori should estabilish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Reference Values

≤ 38 U/L (U/L methylresorufin at 37°C).

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

ANALYTICAL PERFORMANCES

Linearity

Reaction is linear up to a concentration of 250 U/L.
If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L and multiply the result by 10.

Analytical sensitivity

The test sensitivity in terms of detection limit is: 5U/L

"Intra-Assay" precision (within-Run)

Determined on 20 samples for each control (N-H) (Normal-High). Results:

MEAN (U/L)	N = 119	H =215
S.D.	N = 4.13	H = 5.97
C.V.%	N = 3.34	H = 2.78

"Inter-Assay" precision (between-Run)

Determined on 20 samples for each control (N-H) (Normal-High). Results:

MEAN (U/L)	N = 119	H =215
S.D.	N = 5.43	H = 10.7
C.V.%	N = 4.54	H = 5.02

Correlation

A study based comparing this method(y) with a similar method(x) on 50 samples has given a correlating factor **r = 0.997**

y = 0.50054 + 3.9443

Interferences

No interference was observed by the presence of: Bilirubin ≤ 20 mg/dl.
Triglycerides ≤ 300 mg/dl.
Hemoglobin ≤ 150 mg/dl.
A list of drugs and other interfering substances with lipase determination has been reported by Young et. al[2,3].

BIBLIOGRAPHY

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Simbols

CE CE Mark (requirement of 98/79 regulation)

IVD in vitro medical device

LOT Batch Code

Use by

Storage temperature limits

Read instruction for use

Product in Spain

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