Reactivos GPL

Barcelona, España

CE

- Lipase-LQ -

LIPASE

Colorimetric-Kinetic. Liquid

Store at: +2+8ºC.

Presentation:
Cod. EZ025 CONT: R1 4 x 10 + R2 1 x 8 mL + Cal.

Procedure

Quantitative determination of lipase.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY

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 COMPOSITION

R.1 (Buffer)	TRIS pH 8.3 Colipase Desoxycholate Taurodesoxycholate	40 mmol/L. ≥ 1 mg/L. 1.8 mmol/L. 7.2 mmol/L.
R.2 (Substrate)	Tartrate pH 4.0 Lipase Calcium Chloride	15 mmol/L. ≥ 0.7 mmol/L. 0.1 mmol/L.
Lipase Cal	**Calibrator.** Lyophilised human serum**.** The LPS activity (U/L methylresorufin at 37ºC) is indicate on the label.	

PRECAUTIONS

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.

REAGENT PREPARATION AND STABILITY

R.1 – R.2 Ready to use. Stability after opening 90 days at 2-8ºC.
R2 mix gently before use(note1).
Lipase Cal: Dissolve (→) with 1 ml. distilled water. Cap mix gently to dissolve the contents. Stability: 7 days 2-8ºC or 3 months aliquote in small volumes and freeze at –20ºC.
Signs of Reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 580 nm. ≥ 1.4
- R2 is turbid orange coloured micro-emulsion, discard if turning to red.

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

SPECIMEN

Serum or plasma with sodium citrate, EDTA or heparin[1].
Avoid repeated frozen and unfrozen.
Stability: 2 days at 2-8º C.

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 580 nm.
- Thermostatic bath at 37ºC (± 0.1ºC)
- Matched cuvettes 1.0 cm light path.

General laboratory equipment.

TEST PROCEDURE

1. Assay Conditions
- Wavelenght : ..... 580 nm.
- Cuvette: ..... 1 cm light path.
- Constant temperature ..... 37ºC.

2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette(Note 2):

	Blank	Calibrator / Sample
R 1 (mL)	1.0	1.0
R 2 (µL)	200	200
Distilled water (µL)	10	--
Calibrator / Sample (µL)	--	10

4. Mix and incubate at 37º for 1 minute.
5. Read the absorbance (A) of the sample, start the stopwatch and read absorbance at 1 minute interval thereafter for 2 minutes.
6. Calculate the difference of absorbance and the average absorbance difference per minute (ΔA/min.).

CALCULATIONS

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

(ΔA/min) Calibrator - (ΔA/min) Blank= (ΔA/min) net of Calibrator

U/L of lipase in the sample = $\frac{(A)\,Net\ Sample}{(A)\,Net\ Calibrator}$ x Calibrador

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).
Conversion factor: LPS [U/L] x 0,01667= LPS [µkal/L]

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, H Normal and H Pathological (QC003, QC004).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

At 37ºC ≤ 38 u/L. (U/L methylresurofin)
(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE

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.

REAGENT PERFORMANCE

- *Measuring Range*:
  From detection limit of 5 U/L. to linearity limit of 250 U/L., under the described assay conditions.
  If results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L. and multiply result by 10.
- *Precision* :

	Intra-assay n= 20		Inter-assay n= 20	
Mean (U/L)	40,2	59,35	38,5	58,9
SD	0,410	0,875	1,10	1,25
CV	1,02	1,47	2,86	2,13

- *Sensitivity:*
  1 U/L= 0,00059792 (A)
- *Accuracy:*
  Results obtained GPL reagents (y) did not show systematic differences when compared with other commercial reagents (x).
  The results obtained using 101 samples were the following:
  Correlation coefficient (r)[2]: 0.99732
  Regression Equation: y=0.50054x + 3.9443
  The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES

- Triglycerides at 300 mg/dL interfere on determination reducing the activity of enzyme of 6%. Hemoglobin concentration lower than 150 mg/dL and Bilirubin lower than 20 mg/dL do not interfere[2,3,4].
- A list of drugs and other interfering substances with lipase determination has been reported by Young et. al[5,6].

NOTES

1. In some storage conditions (i.e. storage at a temperature lower that the one indicate) a precipitate may appear in the vial that will not influence that the reagent performance; however, it is recommended to resuspend the product with a slight rotation.
2. Use clean disposable pipette tips for its dispensation.

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