Lipase

CYPRESS DIAGNOSTICS

CE

IVD

Enzymatic. Colorimetric. Kinetic
Liquid

Store at 2-8 °C

Configuration

REF	HBE09
VOL	4 x 10 mL
Reagent 1	4 x 10 mL
Reagent 2	1 x 8 mL
Calibrator	1 x Lyoph. - 1 mL

Intended use
The Cypress Diagnostics kit Lipase is an in vitro diagnostic medical device intended to be used for the quantitative measurement of lipase in human serum or plasma. The device is not automated. The measurement of lipase is intended to be used to aid to identify diseases of pancreas such as acute and chronic pancreatitis and obstruction of the pancreatic duct, in patient risk population. This kit is intended to be used by healthcare professionals in a laboratory-based testing environment.
For *in vitro* diagnostic use only. For professional use only.

Clinical significance
Lipase is a pancreatic enzyme which is necessary for the absorption and digestion of nutrients and catalyzes the hydrolysis of glycerol esters of fatty acids. Determination of lipase is used for a 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 sequence of reactions involved in the enzymatic direct lipase determination is the following:

1-2-O-dilauryl-rac-glycero-3-glutaric-(6'-methylresorufin)-ester
$\xrightarrow{Lipase}$ 1-2-O-dilauril-rac-glycerol+glutaric-6'-methylresorufin-ester (not stable)
$\xrightarrow{OH^-}$ Glutaric acid+Methylresorufin

The rate of methylresofurin formation is proportional to the catalytic concentration of lipase.

Reagent composition

Reagent 1	**Buffer** TRIS pH 8,3 (40 mmol/L) Colipase (≥ 1 mg/L) Desoxycholate (1,8 mmol/L) Taurodesoxycholate (7,2 mmol/L)
Reagent 2	**Substrate** Tartrate pH 4,0 (15 mmol/L) Lipase substrate (≥ 0,7 mmol/L) $CaCl_2$ (0,1 mmol/L)
Calibrator	Lyophilized human serum. The lipase activity (U/L methylresorufin at 37 °C) is indicated on the label of the vial.

Precautions
- The calibrator is prepared from human sera, which have been tested and are found to be non-reactive for HBsAg, HCV and HIV (1/2) antibodies. However, all human specimens should be considered potentially infectious.
- All body fluid samples should be considered potentially infectious materials and the appropriate precautions should be taken. Wear personal protective equipment such as gloves, safety glasses, lab coats or aprons when working with possible biohazard contaminants.
- Use Good Laboratory Practices (GLP) when handling this product.
- Please refer to the MSDS, available on our website, for further information.

Preparation
R1 and R2: ready to use. Stability after opening 90 days at 2-8 °C.
R2: mix gently before use[Note 3].
Calibrator: reconstitute the contents of one vial with 1 mL of distilled water. Mix gently until complete solution. Stability: 7 days at 2-8 °C. Divide calibrator solution into small volumes and freeze. Stability: 3 months at -20 °C.

Storage, stability and disposal
All the components of the kit are stable up to the date of expiration as specified on the label, when stored tightly closed, protected from light and contaminations prevented during their use. Storage temperature for this kit is 2-8 °C.
The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 580 nm ≥ 1,4, the reagent should be discarded. R2 is a turbid orange-colored micro-emulsion, discard if turning to red.
Do not use the product if deterioration or contamination is suspected or beyond the expiration date or open container stability period. Dispose unused or deteriorated product and waste in compliance with local regulations.

Additional material required but not provided
- Spectrophotometer or colorimeter. Minimum analyzer specifications:
  Measuring at 580 nm
  Linear measuring range: 0 - 2 AUThermostatic bath at 37 °C (± 0,1 °C)
- Cuvettes, matching the analyzer used (1,0 cm light path)
- Thermostatic bath at 37 °C (± 0,1 °C)
- General laboratory equipment[Note 4]

Samples
Sample type: human serum or plasma
Plasma: with sodium citrate, EDTA or heparin.
Stability: 2 days at 2 - 8 °C.

Procedure
Make sure the reagents and samples are at room temperature.
1. Wavelength 580 nm; Temperature 37 °C; Cuvette (1 cm light path).
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

For Reagent Blank	10 µL Distilled water + 1 mL Reagent 1 + 200 µL Reagent 2
For Calibrator	10 µL Calibrator + 1 mL Reagent 1 + 200 µL Reagent 2
For Sample	10 µL Sample + 1 mL Reagent 1 + 200 µL Reagent 2
Mix will and incubate at 37 °C for 1 minute. Read initial absorbance (A), start the stopwatch and read absorbances every minute for 2 minutes. Calculate the difference between the absorbances and the average absorbance differences per minute (ΔA/min).	

Calculation
Lipase (U/L)

$$= \frac{\Delta A/min\ Sample - \text{ΔA/min Blank}}{\Delta A/min\ Calibrator - \text{ΔA/min Blank}} \times \text{Calibrator lipase activity (U/L)}$$

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 liter of sample (U/L).

Conversion Factor: µkat/L = 0,0167 x U/L

Quality control
Control sera are recommended for the qualification of the reagents and to monitor the performance of assay procedures. Next to legal requirements, we recommend to perform Quality Control on a daily basis and after each calibration.
Use Biochemistry Normal and Pathological Controls Specific (HBC01-S, HBC02-S). If other controls (not manufactured by Cypress) are used, they have to be validated by the user as they can vary. Prepare and measure these controls the same as samples. Measure at least one replicate per control.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Do not continue testing as the results will be invalid. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference values

≤ 38 U/L (≤ 0,63 µkat/L) [methylresorufin at 37 °C]

These values are for orientation purpose. Each laboratory should establish its own reference range.

Performance characteristics
Measuring range: from 5 U/L (detection limit) to 250 U/L (linearity limit). If the obtained results are greater than 250 U/L, dilute the sample 1:10 with saline solution, repeat the determination, and multiply the result by factor 10.

Precision:

	intra-assay (n=20)		inter-assay (n=20)	
Mean (U/L)	40,2	59,35	38,5	58,9
SD	0,41	0,875	1,1	1,25
CV (%)	1,02	1,47	2,86	2,13

Sensitivity: 1 U/L = 0,0006 ΔA/min

Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.
The results of the performance characteristics depend on the analyzer used.

Interferences
Triglycerides at 300 mg/dL interfere on determination reducing the activity of the enzyme with 6%. Hemoglobin up to 150 mg/dL and bilirubin up to 20 mg/dL do not interfere. A list of drugs and other interfering substances with lipase determination has been reported by Young et al.

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Notes

1. For best use of this kit on a Cypress Diagnostics analyzer, we kindly advise to follow the application sheets of the respective analyzer. Please log in to our website (www.diagnostics.be) as a registered user to download the latest application sheets, which are located under the section of the corresponding analyzer. Compatible Cypress analyzers: CYANSmart, CYANStart, CYANExpert 130, CYANVision.
2. In case other instruments (not manufactured by Cypress Diagnostics) are used, the laboratory is responsible to validate the reagents in this kit on those analyzers before testing patient samples.
3. In some storage conditions (lower than the one indicated) a precipitate may appear in the vial that will not influence the reagent performance. However, it is recommended to re-suspend the product with a slight rotation.
4. In order to avoid contamination it is recommended to use disposable material.

Bibliography

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Notice: Any serious incident that has occurred in relation to the device shall be reported to Cypress Diagnostics and the competent authority of the Member State in which the user and/or patient is established.

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