

Lipase

Test principle: Enzymatic colorimetric

chromogenic substrate for lipase $\xrightarrow{\text{lipase}}$ glutaric acid-(6-methylresorufin) ester

glutaric acid-(6-methylresorufin) ester $\xrightarrow{\text{spontaneous}}$ glutaric acid + methylresorufin (red)

This method is based on the cleavage of a specific chromogenic lipase substrate, 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester, emulsified with bile acids. The pancreatic enzyme activity is determined specifically by the combination of bile acid and colipase used in this assay. Virtually no lipase activity is detected in the absence of colipase. Colipase only activates pancreatic lipase, but not other lipolytic enzymes found in serum. The high amount of cholates ensures that the esterases present in the serum do not react with the chromogenic substrate due to the highly negative surface charge.

Enzymatic colorimetric assay with 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methyl-resorufin) ester as substrate. The chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester is cleaved by the catalytic action of alkaline lipase solution to form 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6-methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. Addition of detergent and colipase increases the specificity of the assay for pancreatic lipase.

The color intensity of the red dye formed is directly proportional to the lipase activity. It is determined by measuring the increase in absorbance at 583 nm.

Proposed reagent composition approximately 2+1 formulation

Reagent 1

Composition	Concentration	Catalog Number
Buffer (BICINE, pH 8.0)	50 mmol/l	11 525 778 103
Colipase	0.98 mg/l	10 204 307 103
CaCl ₂	10 mmol/l	
Detergent, such as Na-deoxycholate Taurodeoxycholate (optional)	1.6 mmol/l	11 434 314 103 11 332 686 103
Preservative, such as Sodium azide		

Reagent 2

Composition	Concentration	Catalog Number
Buffer (Tartrate, pH 4.0)	10 mmol/l	
Chromogenic substrate for lipase	0.27 mmol/l	11 034 618 103
Detergent, such as Na-deoxycholate (optional) Taurodeoxycholate	8.8 mmol/l	11 434 314 103 11 332 686 103
Preservative, such as MIT Germall-115, reduced sodium		11 085 905 103 11 276 883 103

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