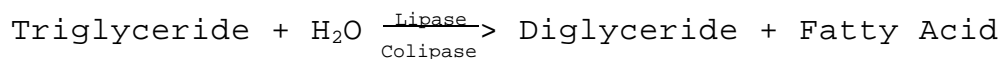


Suitability Assay for COLIPASE

PRINCIPLE:



CONDITIONS: T = 25°C, pH = 8.8, A_{365nm}, Light path = 1 cm

METHOD: Turbidimetric Determination

REAGENTS:

- A. 25 mM Tris HCl Buffer with 25 mM Sodium Deoxycholate, pH 8.8 at 25°C (Deoxycholate)
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, and Deoxycholic Acid, Sodium Salt, Sigma Prod. No. D-6750. Adjust to pH 8.8 at 25°C with 1 M HCl.)
- B. 95% (v/v) Ethanol
(Prepare 20 ml in deionized water using 200 Proof, USP Ethyl Alcohol, Quantum Chemical Corporation.)
- C. 2% (v/v) Triolein Substrate Solution (Triolein)
(Prepare by dissolving 0.5 ml of Triolein(C18:1,[cis]-9, Sigma Prod. No. T-9275, in 25 ml of Reagent B.)
- D. 0.04% (v/v) Substrate Emulsion
(Prepare by adding dropwise (0.05 ml), 1 ml of Reagent C to 50 ml of Reagent A. The resulting emulsion should have approximately 100 absorbance units when measured at 365 nm.)
- E. Lipase Enzyme Solution (Lipase)
(Immediately before use, prepare a solution containing 1 mg/ml of Lipase, Sigma Prod. No. L-0382, in cold deionized water. Then dilute with cold deionized water to approximately 2000 units/ml.)
- F. Colipase Solution¹ (Colipase)
(Immediately before use, prepare a solution containing 0.5 - 1.0 mg/ml in cold deionized water.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent D (Substrate Emulsion)	1.00	1.00

Equilibrate to 25°C. Then add:

Reagent E (Lipase)	0.05	0.05
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Mix by inversion and monitor the $A_{365\text{nm}}$ for both the Test and Blank using a suitably thermostatted spectrophotometer.

There will be a decrease in $A_{365\text{nm}}$ of approximately 0.03 absorbance unit/minute due to some Colipase present in the lipase.

After 3 - 5 minutes, add:

Reagent F (Colipase)	0.01	-----
Deionized Water	-----	0.01

Immediately mix by inversion and record the decrease in $A_{365\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{365\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATION:

$$\Delta A_{365\text{nm}} \text{ Sample} = \Delta A_{365\text{nm}} \text{ Test} - \Delta A_{365\text{nm}} \text{ Blank}$$

Compare the $\Delta A_{365\text{nm}}$ of the Test Sample to that of a Control Sample. The rates should be similar.

FINAL ASSAY CONCENTRATION:

In a 1.06 ml reaction mix, the final concentrations are 23 mM Tris, 23 mM sodium deoxycholate, 0.038% (v/v) triolein, 1.8% (v/v) ethanol, 100 units lipase, 5 - 10 μg colipase.

REFERENCE:

Junge, W., and Leybold, K. (1982) *Clinica Chimica Acta* **123**, 293-302

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NOTES:

1. 5 - 10 μg of Colipase will yield a maximum rate of lipase activity under these conditions.
2. This assay is based on the cited reference.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.