SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of LIPASE
(EC 3.1.1.3)
Sigma Prod. No. L-9518 and L9156

PRINCIPLE:

Triglyceride + H_2O $\xrightarrow{\text{Lipase}}$ Glycerol + 3 Fatty Acids

Glycerol + ATP $\xrightarrow{\text{Glycerokinase}}$ Glycerol-3-P + ADP

Glycerol-3-P + O_2 $\xrightarrow{\text{GPO}}$ Dihydroxyacetone-P + H_2O_2

2 H_2O_2 + 4-AAP + NNDT $\xrightarrow{\text{Peroxidase}}$ Quinoneimine Dye

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
Glycerol-3-P = Glycerol 3-Phosphate
ADP = Adenosine 5'-Diphosphate
GPO = Glycerol-3-Phosphate Oxidase
Dihydroxyacetone-P = Dihydroxyacetone Phosphate
4-AAP = 4-Aminoantipyrine
NNDT = N,N-Diethyl-m-Toluidine

CONDITIONS: T = 37EC, pH = 7.0, A_{545nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 5.0% (v/v) Triton[1] X-100 Solution (X-100)
(Prepare 100 ml in deionized water using Triton[1] X-100, Sigma Stock No. X-100.)

B. 4.0% (w/v) Bovine Serum Albumin (BSA)
(Prepare 100 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-4503 or equivalent.)

C. 100 mM Potassium Phosphate Buffer, pH 7.0 at 37EC
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 37EC with 1 M KOH.)

REAGENTS: (continued)

D. 11% (w/v) Olive Oil Emulsion (Olive Oil)
(Prepare by adding 5 g of Olive Oil, Sigma Prod. No. O-1500 to 5 ml of Reagent A. Sonicate the mixture.[2] To the oil emulsion add 25 ml of Reagent B and 15 ml of Reagent C.)

E. 50 mM MES NaOH Buffer, 6.5 at 37EC
(Prepare 100 ml in deionized water using MES, Free Acid, Sigma Prod. No. M-8250. Adjust to pH 6.5 at 37EC with 1 M NaOH.)

F. 200 mM Trichloroacetic Acid Solution (TCA)
(Prepare 50 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, Sigma Stock No. 490-10.)

G. N,N-Diethyl-m-Toluidine (NNDT)
(Use N,N-Diethyl-m-Toluidine, Eastman Kodak Prod. No. 3454.)

H. 4-Aminoantipyrene (4-AAP)
(Use 4-Aminoantipyrene, Free Base, Sigma Prod. No. A-4382.)

I. Adenosine 5'-Triphosphate (ATP)
(Use Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)

J. Magnesium Chloride ($MgCl_2$)
(Use Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

K. Glycerokinase (GK)
(Use Glycerokinase, Sigma Prod. No. G-4509.)

L. Peroxidase (POD)
(Use Peroxidase, Sigma Prod. No. P-8250.)

M. Glycerol-3-Phosphate Oxidase (GPO)
(Use Glycerol-3-Phosphate Oxidase, Sigma Prod. No. G-9637.)

REAGENTS: (continued)

N. Color Reagent (CR)
(Prepare 200 ml in Reagent E (MES) by dissolving the following products in this order: 4.0 ml of Reagent A (Triton), 0.04 ml of Reagent G (NNDT), 4.0 mg of Reagent H (4-AAP), 24.2 mg of Reagent I (ATP), 40.7 mg of Reagent J ($MgCl_2$), 200 units of Reagent K (GK), 500 units of Reagent M (GPO) and 300 units of Reagent L (POD).)

O. 20 mM Potassium Phosphate Buffer with 2 mM Magnesium Chloride and 0.5 mM Ethylenediaminetetraacetic Acid, pH 7.5 at 37EC
(Prepare 25 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, Magnesium Chloride, Hexahydrate, Sigma Prod. NO. M-0250, and Ethylenediaminetetraacetic Acid, Trisodium Salt, Sigma Stock No. ED3SS. Adjust to pH 7.5 at 37EC with 1 M KOH.)

P. Lipase Enzyme Solution (Lipase)
(Immediately before use, prepare a solution containing 0.7 - 1.4 units/ml of Lipase in cold Reagent O.)

PROCEDURE:

Step 1:

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

	Test	Blank
Reagent D (Olive Oil)	2.00	2.00

Equilibrate to 37EC. Then add:

Reagent P (Lipase)		0.20 -------

Mix by inversion and incubate for exactly 15 minutes. Then add:

Reagent F (TCA)	2.00	2.00
Reagent P (Lipase)		------ 0.20

Mix by inversion and filter the solutions through Whatman No. 42 filter paper.

PROCEDURE: (continued)

Step 2:

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

	Test	Blank
Test Filtrate	0.05	------
Blank Filtrate	------	0.05
Reagent N (CR)	3.00	3.00

Mix by inversion and incubate at 37EC for 15 minutes. Transfer to suitable cuvettes and record the A_{545nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATION:

$$\text{Units/mg enzyme} = \frac{(A_{545nm}\ \text{Test} - A_{545nm}\ \text{Blank})\ (3.05)\ (4.20)}{(15)\ (28.2)\ (0.5)\ (0.05)\ (\text{mg enzyme/RM})}$$

4.20 = Total volume (in milliliters) of Step 1
3.05 = Volume (in milliliters) of Colorimetric Assay in Step 2
15 = Time of assay (in minutes) as per the Unit Definition
28.2 = Millimolar extinction coefficient of Quinoneimine Dye at 545 nm under the assay conditions
0.5 = Conversion factor based on one mole of H_2O_2 produces half a mole of Quinoneimine Dye
0.05 = Volume from Step 1 used in Step 2
RM = Reaction Mix

UNIT DEFINITION:

One unit will produce 1.0 ìmole of glycerol from a triglyceride per minute at pH 7.0 at 37EC in the presence of bovine serum albumin. Assayed in a coupled system with glycerokinase.

FINAL ASSAY CONCENTRATION:

In a 2.20 ml reaction mix, the final concentrations are 32 mM potassium phosphate, 10% (w/v) olive oil, 0.50% (v/v) Triton X-100, 0.05 mM ethylenediaminetetraacetic acid, 2.0% (w/v) bovine serum albumin, 0.14 - 0.28 unit lipase.

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NOTES:

1. Triton is a registered trademark of Union Carbide.

2. Sonicate at 300 watts, for 3 minutes (do this 5 times).

3. Glycerokinase Unit Definition: One unit will convert 1.0 ìmole of glycerol and ATP to L-á-glycerophosphate and ADP per min at pH 9.8 at 25EC is a coupled system with PK/LDH.

4. Glycerol-3-Phosphate Oxidase Unit Definition: One unit will oxidize 1.0 ìmole of L-glycerol 3-phosphate to dihydroxyacetone phosphate with the formation of H_2O_2 per minute at 37EC at the appropriate pH.

5. Peroxidase Unit Definition: One unit will form 1.0 mg purpurogallin from pyrogallol in 20 sec at pH 6.0 at 20EC.

6. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.