



## Product Information

### SIGMA QUALITY CONTROL TEST PROCEDURE

#### Enzymatic Assay of LIPASE

(EC 3.1.1.3)

Sigma Prod. No. L-9518 and L9156

#### PRINCIPLE:

Triglyceride + H<sub>2</sub>O  $\xrightarrow{\text{Lipase}}$  Glycerol + 3 Fatty Acids

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

Glycerol-3-P + O<sub>2</sub>  $\xrightarrow{\text{GPO}}$  Dihydroxyacetone-P + H<sub>2</sub>O<sub>2</sub>

2 H<sub>2</sub>O<sub>2</sub> + 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 = 37°C, pH = 7.0, A<sub>545nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

#### REAGENTS:

- A. 5.0% (v/v) Triton<sup>1</sup> X-100 Solution (X-100)  
(Prepare 100 ml in deionized water using Triton<sup>1</sup> 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 37°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 37°C with 1 M KOH.)

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**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.<sup>2</sup> 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 37°C  
(Prepare 100 ml in deionized water using MES, Free Acid, Sigma Prod. No. M-8250. Adjust to pH 6.5 at 37°C 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<sub>2</sub>)  
(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.)

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**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<sub>2</sub>), 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 37°C  
(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 37°C 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:

	<u>Test</u>	<u>Blank</u>
Reagent D (Olive Oil)	2.00	2.00

Equilibrate to 37°C. Then add:

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

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**PROCEDURE:** (continued)

Step 2:

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

	<u>Test</u>	<u>Blank</u>
Test Filtrate	0.05	-----
Blank Filtrate	-----	0.05
Reagent N (CR)	3.00	3.00

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

**CALCULATION:**

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

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<sub>2</sub>O<sub>2</sub> 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 μmole of glycerol from a triglyceride per minute at pH 7.0 at 37°C 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  $\mu$ mole of glycerol and ATP to L- $\alpha$ -glycerophosphate and ADP per min at pH 9.8 at 25 $^{\circ}$ C is a coupled system with PK/LDH.
4. Glycerol-3-Phosphate Oxidase Unit Definition: One unit will oxidize 1.0  $\mu$ mole of L-glycerol 3-phosphate to dihydroxyacetone phosphate with the formation of H<sub>2</sub>O<sub>2</sub> per minute at 37 $^{\circ}$ C 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 20 $^{\circ}$ C.
6. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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