SIGMA QUALITY CONTROL TEST PROCEDURE

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

PRINCIPLE:

Triglyceride + H₂O $\xrightarrow{\text{Lipase}}$ Glycerol + 3 Fatty Acids

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

Glycerol-3-P + O₂ $\xrightarrow{\text{GPO}}$ Dihydroxyacetone-P + H₂O₂

2 H₂O₂ + 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, A₅₄₅nm, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 5.0% (v/v) Triton X-100 Solution (X-100)
   (Prepare 100 ml in deionized water using Triton 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.)
Enzymatic Assay of LIPASE  
(EC 3.1.1.3)  
Sigma Prod. No. L-9518 and L9156

**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.  
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-Aminoantipyrine (4-AAP)  
(Use 4-Aminoantipyrine, 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 (MgCl₂)  
(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.)
Enzymatic Assay of LIPASE
(EC 3.1.1.3)
Sigma Prod. No. L-9518 and L9156

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 (MgCl₂), 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:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Olive Oil)</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:

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

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (TCA)</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent P (Lipase)</td>
<td>-----</td>
</tr>
</tbody>
</table>

Mix by inversion and filter the solutions through Whatman No. 42 filter paper.
Enzymatic Assay of LIPASE  
(EC 3.1.1.3)  
Sigma Prod. No. L-9518 and L9156

PROCEDURE: (continued)

Step 2:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Filtrate</td>
<td>0.05</td>
<td>------</td>
</tr>
<tr>
<td>Blank Filtrate</td>
<td>------</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent N (CR)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

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

CALCULATION:

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

- 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$_2$O$_2$ 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 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.
Enzymatic Assay of LIPASE  
(EC 3.1.1.3)  
Sigma Prod. No. L-9518 and L9156

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 imole of glycerol and ATP to L-á-glycerophosphate and ADP per min at pH 9.8 at 25°C is a coupled system with PK/LDH.

4. Glycerol-3-Phosphate Oxidase Unit Definition: One unit will oxidize 1.0 imole of L-glycerol 3-phosphate to dihydroxyacetone phosphate with the formation of H₂O₂ per minute at 37°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°C.

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

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.