



SIGMA QUALITY CONTROL TEST
PROCEDURE

Product Information

**Enzymatic Assay of LIPOPROTEIN LIPASE
(EC 3.1.1.34)
Sigma Prod. Nos. L-2254 and L-9656**

PRINCIPLE:



Abbreviations used:

PNPB = p-Nitrophenyl Butyrate

LPL = Lipoprotein Lipase

CONDITIONS: T = 37°C, pH = 7.2, A_{400nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Sodium Phosphate Buffer with 150 mM Sodium Chloride and 0.5% (v/v) Triton¹ X-100, pH 7.2 at 37°C.
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751, Sodium Chloride, Sigma Prod. No. S-9625 and Triton¹ X-100, Sigma Stock. No. X-100. Adjust to pH 7.2 at 37°C with 1 M NaOH.)
- B. Acetonitrile
(Use Acetonitrile, Sigma Prod. No. A-3396.)
- C. 50 mM p-Nitrophenyl Butyrate (PNPB)
(Prepare 1.0 ml in Reagent B using p-Nitrophenyl Butyrate, Sigma Prod. No. N-9876.)
- D. Lipoprotein Lipase Enzyme Solution
(Immediately before use, prepare a solution containing 60 - 70 units/ml of Lipoprotein Lipase in cold Reagent A.)

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PROCEDURE:

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

| | <u>Test</u> | <u>Blank</u> |
|-----------------------------|-------------|--------------|
| Reagent A (Buffer) | 0.90 | 0.90 |
| Reagent D (Enzyme Solution) | 0.10 | 0.10 |

Mix by inversion and equilibrate to 37°C. Monitor the $A_{400\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| | | | |
|------------------|-------|-------|-------|
| Reagent C (PNPB) | 0.010 | ----- | |
| Deionized Water | | ----- | 0.010 |

Immediately mix by inversion and record the increase at $A_{400\text{nm}}$ for approximately 5 minutes.² Obtain the $\Delta A_{400\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{400\text{nm}}/\text{min Test} - \Delta A_{400\text{nm}}/\text{min Blank})(1.01)(\text{df})}{(0.0148)(0.1)}$$

1.01 = Volume (in milliliters) of assay

df = Dilution factor

0.0148 = Micromolar extinction coefficient³ of p-Nitrophenol at 400 nm

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will release 1.0 nanomole (10^{-9} mole) of p-nitrophenol per minute at pH 7.2 at 37°C using p-nitrophenyl butyrate as substrate.

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FINAL ASSAY CONCENTRATION:

In a 1.01 ml reaction mix the final concentrations are 99 mM sodium phosphate, 149 mM sodium chloride, 0.5% (v/v) Triton X-100, 0.50 mM p-nitrophenyl butyrate, 1% (v/v) acetonitrile and 6 - 7 units lipoprotein lipase.

REFERENCES:

Quinn, D.M., Shirai, K., Jackson, R.L., and Harmony, J.K., (1982) *Biochemistry* **21**, 6872-6879

Shirai, K. and Jackson, R. L. (1982) *Journal of Biological Chemistry* **257**, 1253-1258

NOTE:

1. Triton X-100 is a registered trademark of the Rohm and Haas Co.
2. The reaction is linear up to a ΔA_{400nm} /minute of 0.1.
3. The extinction coefficient is described in Quinn, D.M. et al. (1982).
4. This assay is based on the cited references.
5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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