SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of LIPOPROTEIN LIPASE (EC 3.1.1.34)

PRINCIPLE:

PNPB + H_2O $\xrightarrow{LPL}$ p-Nitrophenol + Butyric Acid

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) Triton[1] 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 Triton[1] 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:

	Test	Blank
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_{400nm} 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_{400nm} for approximately 5 minutes.[2] Obtain the ΔA_{400nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

1.01 = Volume (in milliliters) of assay
df = Dilution factor
0.0148 = Micromolar extinction coefficient[3] 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.