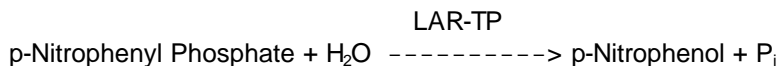


Enzymatic Assay of LAR-PROTEIN TYROSINE PHOSPHATASE

PRINCIPLE:



Abbreviations:

LAR-TP = LAR-Protein Tyrosine Phosphatase

P_i = Inorganic phosphate

CONDITIONS: T = 30°C, pH = 7.0, A_{405nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 50 mM Imidazole HCl Buffer with 100 mM p-Nitrophenyl Phosphate, 5 mM Ethylenediaminetetraacetic acid, 100 mM Sodium Chloride, 10 mM Dithiothreitol and 0.02% (w/v) Bovine Serum Albumin, pH 7.0 at 30°C (2x Reaction Buffer)
(Prepare 5 ml in deionized water using Imidazole, Sigma Prod. No. I-0125, Sigma 104 Phosphatase Substrate, Sigma Stock No. 104-0, Sodium Chloride, Sigma Prod. No. S-9625, DL-Dithiothreitol, Sigma Prod. No. D-0632, Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS, and Albumin, Bovine, Sigma Prod. No. A-8022. Adjust to pH 7.0 at 30°C with 1 M HCl.)
- B. 10 mM Imidazole HCl Buffer, pH 7.0 at 30°C (Enz Dil)
(Prepare 50 ml in deionized water using Imidazole, Sigma Prod. No. I-0125. Adjust to pH 7.0 at 25°C with 1 M HCl.)
- C. 200 mM Sodium Hydroxide Solution (NaOH)
(Prepare 25 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Prod. No. S-5881.)

**Enzymatic Assay of
LAR-PROTEIN TYROSINE PHOSPHATASE**

REAGENTS:

- D. LAR-Protein Tyrosine Phosphatase Enzyme Solution
(Immediately before use, prepare a solution containing 20-40 units/ml of LAR-Protein Tyrosine Phosphatase in cold Reagent B.)

PROCEDURE:

Pipette (in milliliters) the following reagents into a suitable Eppendorf tube:

	<u>Test</u>	<u>Blank</u>
Reagent B (Enz Dil)	-----	0.10
Reagent A (2X Reaction Buffer)	0.10	0.10

Equilibrate to 30°C. Then add:

Enzyme Solution	0.10	-----
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Immediately mix by swirling and incubate at 30°C for exactly 10 minutes. Then add:

Reagent C (NaOH)	0.80	0.80
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Mix by inversion and record the A_{405nm} for both the Test and Blank in a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{405nm} \text{ Test} - A_{405nm} \text{ Blank})(1.0)(df)}{(0.018)(10)(0.1)}$$

1.0 = Total volume (in milliliter) of assay

df = Dilution factor

0.018 = Micromolar extinction coefficient of p-Nitrophenol at 405 nm

10 = Time (in minutes) of assay

0.1 = Volume (in milliliter) of enzyme used

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

**Enzymatic Assay of
LAR-PROTEIN TYROSINE PHOSPHATASE**

CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 nmole of p-nitrophenyl phosphate per minute at pH 7.0 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 0.20 ml reaction mix, the final concentrations are 30 mM imidazole, 2.5 mM ethylenediaminetetraacetic acid, 5 mM dithiothreitol, 50 mM sodium chloride, 0.01% (w/v) bovine serum albumin, 50 mM p-nitrophenyl phosphate, and 2 - 4 unit LAR-protein tyrosine phosphatase.

REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U.) Vol. I, 2nd ed., 495-496, Academic Press, Inc., New York, NY

Cho, H., Ramer, S.E., Itoh, M., Winkler, D.G., Kitas, E., Bannwarth, W., Burn, P., Saito, H., and Walsh, C.T., (1991) *Biochemistry*, 30, 6210-6216

NOTES:

1. The reaction conditions presented, refer to the substrate tested. Dephosphorylation of other substrates may require different conditions.
2. This assay is based on the cited references.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.