Enzymatic Assay of THYMIDINE PHOSPHORYLASE (EC 2.4.2.4)

PRINCIPLE:

$$\text{Thymidine} + \text{Orthophosphate} \xrightarrow{TP} \text{Thymine} + \text{2-Deoxy-R-P}$$

Abbreviations used:
TP = Thymidine Phosphorylase
2-Deoxy-R-P = 2-Deoxy-D-Ribose 1-Phosphate

CONDITIONS: T = 25°C, pH = 7.4, A_{290nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Potassium Phosphate Buffer, pH 7.4 at 25°C.
(Prepare 500 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.4 at 25°C with 1 M NaOH.)

B. 1 mM Thymidine Solution
(Prepare 100 ml in Reagent A using Thymidine, Sigma Prod. No. T-9250.)

C. 10 mM Potassium Phosphate Buffer, pH 7.0 at 25°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod, No. P-5379. Adjust to pH 7.0 at 25°C with 1 M NaOH.)

D. Thymidine Phosphorylase Enzyme Solution
(Immediately before use, prepare a solution containing 1 - 2 units/ml of Thymidine Phosphorylase in cold Reagent C.)

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PROCEDURE:

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

	Test	Blank
Reagent B (Thymidine)	3.00	3.00

Equilibrate to 25°C. Monitor the A_{290nm} until constant[1], using a suitably thermostatted spectrophotometer. Then add:

Reagent C (Enzyme Diluent)	------	0.03
Reagent D (Enzyme Solution)	0.03	------

Immediately mix by inversion and record the decrease in A_{290nm} for approximately 5 minutes. Obtain the ΔA_{290nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{290nm}/\text{min Test} - \Delta A_{290nm}/\text{min Blank})(3.03)(df)}{(1.0)\ (0.03)}$$

3.03 = Total volume (in milliliters) of assay
df = Dilution factor
1.0 = Difference in the millimolar extinction coefficient between thymidine and thymine under the assay conditions
0.03 = Volume (in milliliters) of enzyme used

UNIT DEFINITION[2]:

One unit will convert 1.0 μmole each of thymidine and phosphate to thymine and 2-deoxyribose 1-phosphate per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.03 ml reaction mix, the final concentrations are 198 mM potassium phosphate, 1 mM thymidine and 0.03 - 0.06 unit thymidine phosphorylase.

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REFERENCE:

Krenitsky, T.A. and Bushby, S.R.M. (1979) *United States Patent* 4,178,212, 1-8, Burroughs Wellcome Co., Research Triangle Park, NC

NOTES:

1. The initial A_{290nm} should be between 1.8 and 1.95. If the absorbance is not within the specific range, then fresh reagents should be prepared.

2. One Sigma unit is equivalent to 0.614 International Unit.

3. This assay is based on the cited reference.

4. 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.