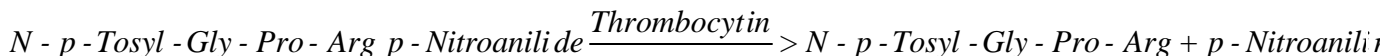


Enzymatic Assay of THROMBOCYTIN

PRINCIPLE:



CONDITIONS: T = 37°C, pH 8.4, $A_{405\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris and 100 mM Imidazole Buffer with 150 mM Sodium Chloride, pH 8.4 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503, Imidazole, Prod. No. I-0250, and Sodium Chloride, Prod. No. S-9625. Adjust to pH 8.4 at 37°C with 1 M HCl.)
- B. 1.5 mM N-p-Tosyl-Gly-Pro-Arg p-Nitroanilide Solution (Substrate)
(Prepare 5 ml in deionized water using N-p-Tosyl-Gly-Pro-Arg p-Nitroanilide, Acetate, Prod. No. T-1637.)
- C. 0.1% (w/v) Bovine Serum Albumin (BSA)
(Prepare 10 ml in Reagent A.)
- D. Thrombocytin Enzyme Solution
(Immediately before use, prepare a solution containing 50 units/ml of Thrombocytin in Reagent C.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.55	2.55
Reagent B (Substrate Solution)	0.30	0.30

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PROCEDURE: (continued)

Equilibrate to 37°C. Monitor the $A_{405\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent D (Enzyme Solution)	0.15	-----
Reagent C (BSA)	-----	0.15

Immediately mix by inversion and record the increase in $A_{405\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{405\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{\Delta A_{405\text{nm}}/\text{min Test} - \Delta A_{405\text{nm}}/\text{min Blank}}{(0.0106) (\text{mg enzyme/ml RM})}$$

0.0106 = Micromolar extinction coefficient of
p-nitroaniline at 405nm

RM = Reaction Mix

UNIT DEFINITION:

One unit will release 1.0 nmole of p-nitroaniline from N-p-tosyl-Gly-Pro-Arg p-nitroanilide per minute at pH 8.4 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 90 mM Tris, 90 mM imidazole, 135 mM sodium chloride, 0.005% BSA, 0.15 mM N-p-tosyl-Gly-Pro-Arg p-nitroanilide.

REFERENCES:

Kirby, E.P. (1979) *Biochemistry*, **18**, 3564

NOTES:

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

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