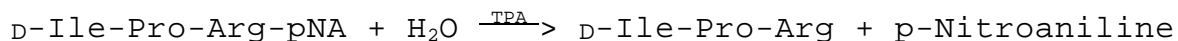


**Enzymatic Assay of TISSUE PLASMINOGEN ACTIVATOR
SINGLE CHAIN T-PA
Amidolytic Activity**

PRINCIPLE:



Abbreviation used:

pNA = p-Nitroanilide

CONDITIONS: T = 25°C, pH 6.0 and 9.0, A_{405nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 500 mM Potassium Phosphate Buffer, pH 6.0 at 25°C
(Phos Buffer)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.0 at 25°C with 1 M NaOH.)
- B. 500 mM Tris HCl Buffer, pH 9.0 at 25°C (Tris Buffer)
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 9.0 at 25°C with 1 M HCl.)
- C. 0.40 mM D-Ile-Pro-Arg p-Nitroanilide Solution
(Substrate pH 6)
(Prepare 20 ml in Reagent A using D-Ile-Pro-Arg p-Nitroanilide Dihydrochloride, Sigma Prod. No. I-0898.)
- D. 0.40 mM D-Ile-Pro-Arg p-Nitroanilide Solution
(Substrate pH 9)
(Prepare 20 ml in Reagent B using D-Ile-Pro-Arg p-Nitroanilide Dihydrochloride, Sigma Prod. No. I-0898.)
- E. 1 M Potassium Bicarbonate Solution (Enzyme Diluent)
(Prepare 25 ml in deionized water using Potassium Bicarbonate, Sigma Prod. No. P-9144.)
- F. Tissue Plasminogen Activator Single Chain T-PA Enzyme Solution (T-PA)
(Immediately before use, prepare a solution containing 0.5 -1.0 unit/ml of Tissue Plasminogen Activator)

Single Chain T-PA in cold Reagent E.)

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PROCEDURE:

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

	<u>Test 1</u>	<u>Blank 1</u>	<u>Test 2</u>	<u>Blank 2</u>
Reagent C (Substrate pH 6)	0.90	0.90	-----	-----
Reagent D (Substrate pH 9)	-----	-----	0.90	0.90
Reagent F (T-PA)	0.10	-----	0.10	-----
Reagent E (Enzyme Diluent)	-----	0.10	-----	0.10

Immediately mix by inversion and record the increase in $A_{405\text{nm}}$ for approximately 10 minutes using a suitably thermostatted spectrophotometer. Obtain the $\Delta A_{405\text{nm}}/\text{minute}$ using the maximum linear rate for both the Tests and Blanks.¹

Compare the activity of Test 2 to that of a control sample. The activity should be similar.

CALCULATIONS:

$$\text{Units/ml} = \frac{(\Delta A_{405})(df)(1)}{(0.1)(10)}$$

1 = Volume (in milliliter) of assay

df = Dilution factor

0.1 = (Volume (in milliliter) of enzyme used

10 = Millimolar extinction coefficient of p-nitroaniline

at 405 nm under the conditions of the assay

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix the final concentrations are 0.36 mM p-Ile-Pro-Arg p-nitroanilide, 450 mM Tris or 450 mM potassium phosphate, 100 mM potassium bicarbonate, and 0.05 - 0.10 unit tissue plasminogen activator single chain T-PA.

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REFERENCE:

Verheijen, J.H., Kluft, C. Chang, G.T.G. and Mullaart, E. (1984) in *Methods of Enzymatic Analysis* (Bergmeyer, J. and Grassl, M. eds), 425-433, Volume 5, Verlag Chemie, Deerfield Beach, Florida

Friberger, P., Knös, M., Gustavsson, S., Aurell, L., and Claeson, G. (1978) *Haemostasis*, 7, 138-145

NOTES:

1. The assay is run at pH 6.0 and pH 9.0. There should be very little activity observed at pH 6.0 with the maximum activity observed at pH 9.0.
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.