

## Enzymatic Assay of DIPEPTIDYL PEPTIDASE (EC 3.4.14.5)

### PRINCIPLE:

Gly-Pro-4-Nitroanilide + H<sub>2</sub>O  $\xrightarrow{\text{Dipeptidyl Peptidase}}$  Gly-Pro + p-Nitroaniline

**CONDITIONS:** T = 37°C, pH = 8.0, A<sub>405nm</sub>, Inc. = 15 minutes

**METHOD:** Stopped Rate Spectrophotometric Determination using a Microplate Reader

### REAGENTS:

- A. 0.1 M Tris, pH 8.0 at 37EC  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 37°C using 1 M HCl.)
- B. 1 mM Gly-Pro-pNA Solution  
(Prepare 10 ml in Reagent A, using Gly-Pro-p-nitroaniline, Hydrochloride, Sigma Prod. No. G-0513. **Prepare Fresh.**)
- C. 1 mM p-Nitroaniline Solution (pNA)  
(Prepare 10 ml in Reagent A, using p-Nitroaniline, Sigma Prod. No. N-2128. **Prepare Fresh.**)
- D. Dipeptidyl Peptidase Enzyme Solution  
(Immediately before use prepare the following in cold reagent A. Reconstitute 0.75 unit vial in 1 ml. Dilute to obtain 0.04 - 0.08 units/ml.)

### PROCEDURE:

1. Set up microtitre 96-well plate with the following standards:

S1 = 20 µl 1 mM pNA (20 nmols)

S2 = 40 µl 1 mM pNA (40 nmols)

S3 = 60 µl 1 mM pNA (60 nmols)

S4 = 80 µl 1 mM pNA (80 nmols)

S5 = 100 µl 1 mM pNA (100 nmols)

Make all wells up to 0.1 ml with Reagent A.

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**PROCEDURE:**

2. Pipette in enzyme samples as follows:

T1 = 10  $\mu$ l

T2 = 20  $\mu$ l

T3 = 30  $\mu$ l

T4 = 40  $\mu$ l

T5 = 50  $\mu$ l

Make all wells up to 0.1 ml with Reagent A.

3. Add 0.1 ml of Reagent B to each well (including standards) to start the reaction.
4. Incubate at 37°C for 15 minutes.
5. Read absorbance at 405 nm in a microplate reader.

**CALCULATIONS:**

Plot a standard curve of A405nm versus nmoles of pNA. Calculate nmoles/minute and hence from this determine  $\mu$ moles/min/ml of enzyme.

**UNIT DEFINITION:**

One unit produces 1.0  $\mu$ mole of 4-Nitroaniline from Gly-Pro-4-nitroaniline per minute in 0.1 M Tris/HCl at pH 8.0 at 37°C.

**FINAL ASSAY CONCENTRATION:**

In a 0.2 ml reaction mix, the final concentrations are 100 mM Tris, 0.5 mM Gly-Pro-pNA, and varying amounts of enzyme.

**REFERENCES:**

1. This assay is based on the cited reference.
2. 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.**