## Enzymatic Assay of CATHEPSIN G
## (EC 3.4.21.20)

**PRINCIPLE:**

N-Succinyl-Ala-Ala-Pro-Phe p-NA + $H_2O$ $\xrightarrow{\text{Cathepsin G}}$ N-Succinyl-Ala-Ala-Pro-Phe + p-nitroaniline

Abbreviation used:
p-NA = p-nitroanilide

**CONDITIONS:** T = 37°C, pH = 7.5, $A_{410nm}$, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 mM HEPES NaOH Buffer, pH 7.5 at 37EC
(Prepare 100 ml in deionized water using HEPES, Free Acid, Sigma Prod. No. H-3375. Adjust to pH 7.5 at 37°C with 1 M NaOH.)

B. Dimethyl Sulfoxide (DMSO)
(Use Dimethyl Sulfoxide, Sigma Prod. No. D-5879.)

C. 20 mM N-Succinyl-Ala-Ala-Pro-Phe p-Nitroanilide Solution (Substrate)
(Prepare 2 ml in Reagent B using N-Succinyl-Ala-Ala-Pro-Phe p-Nitroanilide, Sigma Prod. No. S-7388.)

D. Cathepsin G Enzyme Solution
(Immediately before use, prepare a solution containing 1.25 - 2.50 units/ml of Cathepsin G in cold deionized water.)

**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Reagent A (Buffer) | 1.60 | 1.60 |
| Reagent C (Substrate) | 0.20 | 0.20 |

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**PROCEDURE:** (continued)

Mix by inversion and equilibrate to 37°C. Then add:

| | Test | Blank |
|---|---|---|
| Reagent D (Enzyme Solution) | 0.025 | ------ |
| Deionized Water | ------ | 0.025 |

Immediately mix by inversion and record the increase in $A_{410nm}$ for approximately 5 minutes. Obtain the $\Delta A_{410nm}$/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{410nm}/\text{min Test} - \Delta A_{410nm}/\text{min Blank})(1000)(1.825)(df)}{(8.8)(0.025)(60)}$$

1000 = Factor for converting µmoles to nanomoles as per the Unit Definition
1.825 = Total volume (in milliliters) of assay
df = Dilution factor
8.8 = Millimolar extinction coefficient of p-nitroaniline at 410 nm
0.025 = Volume (in milliliters) of enzyme used
60 = Factor for converting minutes to seconds as per the Unit Definition

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

**UNIT DEFINITION:**

One unit will release one nanomole of p-nitroaniline per second from N-Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide at pH 7.5 at 37°C.

**FINAL ASSAY CONCENTRATION:**

In a 1.825 ml reaction mix, the final concentrations are 88 mM HEPES, 2.2 mM N-Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, 11% (v/v) dimethyl sulfoxide and 0.03 - 0.06 unit Cathepsin G.

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**REFERENCE:**

Barrett, A.J. (1981) *Methods in Enzymology* **80**, Part C, 561-565

**NOTES:**

1. The 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.**