

Enzymatic Assay of PHOSPHODIESTERASE II (EC 3.1.16.1)

PRINCIPLE:

RNA-Core $\xrightarrow{\text{Phosphodiesterase II}}$ Acid Soluble Nucleoside-3'-Phosphates

Abbreviation used:

RNA = Ribonucleic Acid

CONDITIONS: T = 37°C, pH = 6.5, $A_{260\text{nm}}$, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 17 mg/ml Ribonucleic Acid-Core Solution (RNA Solution)
(Prepare in deionized water using Ribonucleic Acid, Core, Sigma Prod. No. R-6875.)
- B. 250 mM Succinic Acid Sodium Salt pH 6.5 at 37°C (Succinate Buffer)
(Prepare in deionized water using Succinic Acid, Disodium Salt, Hexahydrate, Sigma Prod. No. S-2378. Adjust the pH to 6.5 at 37°C using 1 M HCl.)
- C. 0.25% (w/v) Uranyl Acetate with 250 mM Perchloric Acid Solution (UA-PA Solution)
(Prepare 50 ml in deionized water using Uranyl Acetate, Dihydrate, Fluka Stock No. 94260 and Perchloric Acid, Sigma Stock No. 24425-2.)
- D. Phosphodiesterase II Solution (Enzyme Solution)
(Immediately before use prepare a solution containing 3 - 6 units/ml of Phosphodiesterase II in cold deionized water.)

**Enzymatic Assay of PHOSPHODIESTERASE II
(EC 3.1.16.1)**

PROCEDURE:

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

| | <u>Test</u> | <u>Blank</u> |
|------------------------------|-------------|--------------|
| Reagent A (RNA Solution) | 0.50 | 0.50 |
| Reagent B (Succinate Buffer) | 0.40 | 0.40 |
| Deionized Water | 0.90 | 1.10 |

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

| | | |
|-----------------------------|------|-------|
| Reagent D (Enzyme Solution) | 0.20 | ----- |
|-----------------------------|------|-------|

Immediately mix by swirling and incubate both tubes at 37°C for exactly 30 minutes. Then add:

| | | |
|----------------------------|------|------|
| Reagent C (UA-PA Solution) | 2.00 | 2.00 |
|----------------------------|------|------|

Immediately mix by inversion and chill in an ice bath for 5 minutes. Centrifuge both tubes. Dilute 0.2 ml of each supernatant to 8.0 ml using deionized water. Read the A_{260nm} of the Test solution against the Blank solution using suitable cuvettes.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{260nm} \text{ Test} - A_{260nm} \text{ Blank})(8)(2)(df)}{(16)(0.2)(0.2)}$$

8 = Volume (in milliliters) of diluted assay

2 = Dilution volume (in milliliters) after the addition of UA-PA

df = Dilution factor

16 = Increase in A_{260nm} at 30 minutes as per the Unit Definition

0.2 = Volume (in milliliter) of enzyme used

0.2 = Volume (in milliliter) of reaction mix used in diluted assay

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

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

Enzymatic Assay of PHOSPHODIESTERASE II (EC 3.1.16.1)

UNIT DEFINITION:

One unit will produce acid soluble nucleotides equivalent to a ΔA_{260} of 16 in 30 minutes at pH 6.5 at 37°C, in a 2.0 ml reaction mixture.

FINAL ASSAY CONCENTRATION:

In a 2.00 ml reaction mix, the final concentrations are 4.25 mg/ml ribonucleic acid-core, 50 mM succinic acid, and 0.6 - 1.2 units phosphodiesterase II.

REFERENCE:

Hilmoe, R.J. (1960) *Journal of Biological Chemistry* **235**, 2117-2121

NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.
2. This assay is based on the cited reference.

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