Enzymatic Assay of NADPH-FMN OXIDOREDUCTASE (EC 1.6.99.1)

PRINCIPLE:

β-NADPH + FMN $\xrightarrow{\text{NADPH-FMN Oxidoreductase}}$ β-NADP + $FMNH_2$

Abbreviations used:
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
FMN = Flavin Mononucleotide
β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
$FMNH_2$ = Flavin Monomucleotide, (Reduced)

CONDITIONS: T = 30°C, pH 7.0, A_{340nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 7.0 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504. Adjust to pH 7.0 at 30°C with 50 mM Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751.)

B. 1.0 mM Flavin Mononucleotide Solution (FMN)
(Prepare 10 ml in Reagent A using Flavin Mononucleotide, Sodium Salt, Sigma Prod. No. F-2253. **PREPARE FRESH.**)

C. 2.0 mM β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (β-NADPH)
(Prepare 5 ml in Reagent A using β-Nicotinamide Adenine Dinucleotide, Phosphate, Reduced Form, Tetrasodium Salt, Sigma Prod. No. N-1630 or dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Sigma Stock No. 201-210, in the appropriate volume of Reagent A. **PREPARE FRESH**.)[1]

D. NADPH-FMN Oxidoreductase Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.20 unit/ml of NADPH-FMN Oxidoreductase in cold Reagent A.)

Enzymatic Assay of NADPH-FMN OXIDOREDUCTASE
(EC 1.6.99.1)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.65	2.75
Reagent B (FMN)	0.05	0.05
Reagent C (β-NADPH)	0.20	0.20

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the decrease in A_{340} for approximately 5 – 10 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}\ \text{Test} - A_{340nm}\ \text{Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm
0.1 = Volume (in milliliter) of enyzme used at 340nm

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

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

UNIT DEFINITION:

One unit will reduce 1.0 μmole of FMN to $FMNH_2$ per minute at pH 7.0 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.02 mM FMN, 0.13 mM β-NADPH and 0.005 - 0.02 unit NADPH-FMN oxidoreductase.

.

Enzymatic Assay of NADPH-FMN OXIDOREDUCTASE (EC 1.6.99.1)

REFERENCES:

Jablonski, E., and DeLuca, M. (1977) *Biochemistry* **16**, 2932-2936

NOTES:

1. Correct NADPH concentrations for purity and water content.
2. This assay is based on the cited reference.
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.