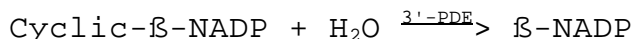


**Enzymatic Assay of 3'-PHOSPHODIESTERASE, 2':3'-CYCLIC  
NUCLEOTIDE  
(EC 3.1.4.37)**

**PRINCIPLE:**



Abbreviations used:

Cyclic- $\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide  
2':3'-Cyclic Monophosphate

3'-PDE = 3'-Phosphodiesterase, 2':3'-Cyclic Nucleotide

$\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form

$\beta$ -NADPH =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Reduced Form

G 6-P = Glucose 6-Phosphate

G-6-PDH = Glucose-6-Phosphate Dehydrogenase

**CONDITIONS:** T = 25°C, pH = 6.00, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 200 mM 2-[N-Morpholino] Ethanesulfonic Acid (MES)  
Buffer, pH 6.00 at 25°C  
(Prepare 200 ml in deionized water using MES, Free  
Acid,  
Prod. No. M-8250. Adjust to pH 6.00 at 25°C with  
1 M KOH.)
- B. 10 mM  $\beta$ -Nicotinamide Adenine Dinucleotide 2':3'-Cyclic  
Monophosphate Solution (Cyclic- $\beta$ -NADP)  
(Prepare 5 ml in Reagent A using  $\beta$ -Nicotinamide  
Adenine Dinucleotide 2':3'-Cyclic Monophosphate,  
Sodium Salt, Prod. No. N-5257. **PREPARE FRESH.**)
- C. 50 mM Glucose 6-Phosphate Solution (G 6-P)  
(Prepare 5 ml in Reagent A using Glucose 6-Phosphate,  
Monosodium, Prod. No. G-7879.)

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**REAGENTS:** (continued)

- D. 300 mM Magnesium Chloride Solution ( $MgCl_2$ )  
(Prepare 5 ml in Reagent A using Magnesium Chloride, Hexahydrate, Prod. No. M-0250.)
- E. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G-6-PDH)  
(Immediately before use, prepare a solution containing 100 - 150 units/ml of Glucose-6-Phosphate Dehydrogenase, Prod. No. G-6378, in cold deionized water.)
- F. 3'-Phosphodiesterase, 2':3'-Cyclic Nucleotide Enzyme Solution  
(Immediately before use, prepare a solution containing 0.2 unit/ml of 3'-Phosphodiesterase, 2':3'-Cyclic Nucleotide in cold deionized water.)

**PROCEDURE:**

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	14.0
Reagent B (Cyclic- $\beta$ -NADP)	2.0
Reagent C (G 6-P)	2.0
Reagent D ( $MgCl_2$ )	2.0

Mix by swirling and equilibrate to 25°C.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	1.00	1.00
Reagent E (G-6-PDH)	0.05	0.05

Mix by inversion and equilibrate to 25°C. Monitor the  $A_{340nm}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Deionized Water	-----	0.05
Reagent F (Enzyme Solution)	0.05	-----

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**PROCEDURE:** (continued)

Immediately mix by inversion and record the increase in  $A_{340\text{nm}}$  for approximately 5 minutes. Obtain the  $\Delta A_{340\text{nm}}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/mg enzyme} = \frac{\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank}}{(6.22) (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of  $\beta$ -NADPH at 340 nm  
RM = Reaction Mix

**UNIT DEFINITION:**

One unit will convert 1.0  $\mu\text{mole}$  of 2':3'-cyclic NADP to NADP per minute at pH 6.0 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 1.10 ml reaction mix, the final concentrations are 182 mM MES, 0.91 mM  $\beta$ -nicotinamide adenine dinucleotide 2':3'-cyclic monophosphate, 4.5 mM glucose 6-phosphate, 27 mM magnesium chloride, 5.0 - 7.5 units glucose-6-phosphate dehydrogenase and 0.01 unit 3'-phosphodiesterase, 2':3'-cyclic nucleotide.

**REFERENCES:**

Sogin, D.C. (1976) *J. Neurochemistry* **27**, 1333-1336.

**NOTES:**

1. Glucose 6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0  $\mu\text{mole}$  of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of NADP at pH 7.4 at 25°C.
2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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