SIGMA QUALITY CONTROL TEST

ProductInformation

Enzymatic Assay of ADP-RIBOSYLCYCLASE

PRINCIPLE:

β-NAD $\xrightarrow{ADPR}$ cyclic ADP-Ribose

Abbreviations used:
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
ADPR = ADP-Ribosylcyclase
cyclic ADP = cyclic Adenosine Diphosphate

CONDITIONS: T = 25°C, pH = 7.0

METHOD: HPLC Analysis of Products

REAGENTS:

A. 20 mM Potassium Phosphate Buffer, pH 7.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 25°C with 1 M KOH.)

B. 6.67 mM β-Nicotinamide Adenine Dinucleotide Solution (β-NAD)
(Prepare 1 ml in Reagent A using β-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-1636. **PREPARE FRESH**.)

C. 1 M Acetic Acid Solution (HOAC)
(Prepare 10 ml in deionized water using Acetic Acid, Glacial, Sigma Prod. No. A-6283.)

D. 10 mM Potassium Phosphate and 500 mM Acetic Acid Solution (Std Dil)
(Prepare 5 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, and Acetic acid, Glacial, Sigma Prod. No. A-6283.)

REAGENTS: (continued)

E. 250 µg/ml Cyclic Adenosine Diphosphate-Ribose Standard Solution (cADP Ribose)
(Prepare 1 ml by dissolving a 250 µg vial of Cyclic Adenosine Diphosphate-Ribose, Sigma Prod. No. C-7323, in Reagent D. To confirm the concentration, add 0.1 ml of this solution to 1 ml of Reagent A and determine the concentration using the extinction coefficient. Centrifuge the remaining solution of C-7323 in an Eppendorf tube at high speed for 10 minutes in a microcentrifuge. This solution is injected into the HPLC instrument as a standard and the concentration of the standard solution should be about 250 µg/ml.[1])

F. 100 mM Ammonium Phosphate Solution
(Prepare 100 ml in deionized water using Ammonium Phosphate, Monobasic, Sigma Prod. No. A-1645.)

G. 25% (v/v) Acetonitrile Solution
(Prepare 100 ml in deionized water using Acetonitrile, Sigma Prod. No. A-3396.)

H. ADP-Ribosylcyclase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of ADP-Ribosylcyclase in cold Reagent A.)

PROCEDURE:

Step 1:

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

		Test	Blank[2]
Reagent B (β-NAD)	0.15	0.15	

Equilibrate to 25°C. Then add:

Reagent H (Enzyme Solution)	0.05	------

Mix by swirling and incubate at 25°C for exactly 5 minutes. Then add:

Reagent C (HOAC)	0.20	0.20
Reagent H (Enzyme Solution)	------	0.05

Mix by swirling and place on ice. Centrifuge the Test and Blank at high speed in a microcentrifuge for 10 minutes. Assay the supernatants for cyclic ADP-ribose by the HPLC assay described in Step 2.

PROCEDURE: (continued)

Step 2:

HPLC Analysis:

Column: Supelcosil LC-18, Supelco Catalog No. 5-8298, 25 mm x 4.6 mm
Injection Volume: 20 µL
Standard: cADP-Ribose (Reagent E)
Flow Rate: 1.5 ml/minute
Wavelength: 254 nm
Attenuation: 8
Buffer A: Ammonium Phosphate (Reagent F)
Buffer B: Acetonitrile (Reagent G)
HPLC Program: Equilibrate the column with 100% Reagent A.

Time (Minutes)	Function	%Buffer B
0.01	Inject Sample	0
10	Buffer B	5
20	Buffer B	15
21	Buffer B	0
31	Stop	

CALCULATION:

$$\text{Units/ml} = \frac{(\text{SPA} \times 10^{-6} - \text{BPA} \times 10^{-6})(0.4)(df)(A)}{(0.05)(0.02)}$$

SPA = Sample Peak Area
BPA = Blank Peak Area
0.4 = Volume (in milliliter) of assay
df = Dilution factor
0.05 = Volume (in milliliter) of enzyme used
0.02 = Injection volume (in milliliter) for HPLC[3]

$$A = \frac{(\text{Concentration of standard µg/ml divided by 540 µg/µmole})(0.02)}{\text{Standard Peak Area} \times 10^{-6}}$$

UNIT DEFINITION:

One unit of ADP ribosylcyclase will produce 1 µmole of cyclic ADP ribose from β-NAD^+ in 5 minutes at 25°C and pH 7.0.

FINAL ASSAY CONCENTRATION:

In a 0.20 ml reaction mix, the final concentrations are 20 mM potassium phosphate, 5 mM β-nicotinamide adenine dinucleotide, and 0.025 - 0.05 unit ADP-ribosylcyclase.

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REFERENCE:

Lee, H.C. and Aarhus, R. (1991) *Cell Regulation* **2**, 203-209

Lee, H.C., Walseth, T.F., Bratt, G.T., Hayes, R.N., and Clapper, D.L. (1989) *J. Biol. Chem.* **264**, 1608-1615

NOTES:

1. Calculate the standard concentrations as follows:
   µg/ml = (A_{254nm}/14.3)(Dilution Factor)(540 µg/µmole).
   14.3 is the EmM for cyclic ADP-Ribose as described in Lee, H.C. et al. (1989).

2. A separate Blank must be run for each Test. Store blanks on ice before adding enzyme.

3. The factor of injection volume can be left out if the sample and standard volumes are the same.

4. This assay is based on the cited references.

5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.