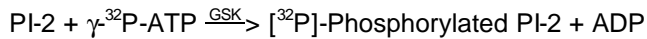


Enzymatic Assay of GLYCOGEN SYNTHASE KINASE 3B

PRINCIPLE:



Abbreviations used:

PI-2 = Phosphatase Inhibitor-2

$\gamma\text{-}^{32}\text{P-ATP}$ = Adenosine 5-Triphosphate $\gamma^{32}\text{-P}$ label

GSK = Glycogen Synthase Kinase 3B

ADP = Adenosine 5'-Triphosphate

CONDITIONS: T = 30°C, pH = 7.5

METHOD: Radioactive

REAGENTS:

- A. 20 mM Tris HCL Buffer, pH 7.5 at Room Temperature (Enz Dil)
(Prepare 10 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.5 at room temperature using 1 M HCl.)
- B. $\gamma\text{-}^{32}\text{P}$ -Adenosine 5-Triphosphate Solution ($\gamma\text{-}^{32}\text{P-ATP}$)
(Use product with a specific activity of 3000 curies/mmol.)
- C. 80 mM Tris HCl Buffer, with 40 mM Magnesium Chloride, 20 mM Dithiothreitol and 0.8 mM Adenosine 5'-Triphosphate, pH 7.5 at room temperature (4X Reaction Buffer)
(Prepare 25 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250, DL-Dithiothreitol, Sigma Prod. No. D-0632, and Adenosine 5-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394. Adjust to pH 7.5 at room temperature with 1 M HCl. Before use, add Reagent B ($\gamma\text{-}^{32}\text{P-ATP}$) to a final concentration of 200 μCi per ml of the 4X Reaction Buffer).
- D. 10 mM Sodium Pyrophosphate Solution
(Prepare 200 ml in deionized water using Sodium Pyrophosphate, Disodium Salt, Anhydrous, Sigma Prod. No. P-8135.)

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REAGENTS:

- E. 10% (w/v) Trichloroacetic Acid Solution (TCA Wash Solution)
(Prepare 200 ml in Reagent D using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- F. Chromatography Paper
(Using Whatman 3 mm chromatography paper, cut into 1 x 2 cm squares.)
- G. Phosphatase Inhibitor-2 Solution (PI-2)
(Immediately before use, reconstitute a 100 µg vial of Phosphatase Inhibitor-2, Sigma Prod. No. P-8218, with 200 µl of deionized water. Determine protein concentration by the Bradford method. Dilute to 0.5 mg/ml.)
- H. Glycogen Synthase Kinase 3B Enzyme Solution
(Immediately before use, reconstitute a vial with 100 µl of deionized water. Further dilute 5 fold in Reagent A a final concentration of 400 units/ml.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent H (Enzyme Solution)	0.005	-----
Reagent A (Enz Dil)	-----	0.005
Reagent G (PI-2)	0.01	0.01
Reagent C (4x Reaction Buffer)	0.005	0.005

Vortex gently for a few seconds and incubate for 10 minutes at 30°C.

Remove a 0.015 ml aliquot from both the Test and Blank reaction mixture and place on Reagent F (1 x 2 cm chrom. paper). Soak the paper rectangles in Reagent E (TCA Wash Solution) at room temperature for 15 minutes. Wash the paper rectangles 4 times with Reagent E. Each wash should consist of 10 ml of Reagent E per paper rectangle. Agitate gently throughout each wash for 15 minutes. This is then followed by a single wash with ethanol and another wash with acetone.

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Dry the paper pieces at room temperature or under a lamp. Then count the radioactivity that has been incorporated into precipitated phosphatase inhibitor-2 using the Cerenkov mode (i.e. count the β -emission without scintillation fluid using the ^3H channel.)

CALCULATIONS:

1. Count **R**, the radioactivity of 5 μl of the 4X Reaction Buffer, in order to obtain the total radioactivity in cpm per assay tube (perform in duplicate).
2. Divide the above value (**R**) by the amount of ATP present in the assay tube (4000 pmol), in order to obtain the specific radioactivity, **SR**. **SR = R/4000 cpm/pmol**.
3. Subtract the blank value from the count of the sample and multiply the result by a factor of 4/3 (to adjust for aliquots taken from the reaction) in order to obtain the total counts per reaction **C**.
C = (C sample - C blank) x 4/3

$$\text{Units/ml} = \frac{(\mathbf{C})(\text{df})}{(\mathbf{SR})(10)(0.005)}$$

df = Dilution factor

10 = Conversion factor to convert to a one minute rate.

0.005 = Volume (in milliliters) of enzyme used

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

UNIT DEFINITION:

One unit will transfer one pmol of phosphate from ATP to phosphate inhibitor 2 per minute at pH 7.5 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 0.020 ml reaction mix, the final concentrations are 25 mM Tris, 10 mM magnesium chloride, 5 mM DL-dithiothreitol, 0.2 mM adenosine 5-triphosphate, 5 μg phosphatase inhibitor P-2 and 2 units glycogen synthase kinase 3B.

REFERENCES:

DePaoli-Roach, A.A. (1984) *Journal of Biological Chemistry*, 259, 12144-12152

Wang, Q.M., Fiol, C.J., DePaoli-Roach, A.A., and Roach, P.J. (1994) *Journal of Biological Chemistry*, 269, 14566-14574

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NOTES:

1. This assay is based on the cited references.
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.