Enzymatic Assay of GLYCOGEN SYNTHASE KINASE 3B

PRINCIPLE:

PI-2 + γ-^{32}P-ATP $\xrightarrow{GSK}$ [^{32}P]-Phosphorylated PI-2 + ADP

Abbreviations used:
PI-2 = Phosphatase Inhibitor-2
γ-^{32}P-ATP = Adenosine 5-Triphosphate γ^{32}-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. γ-^{32}P-Adenosine 5-Triphosphate Solution (γ-^{32}-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 (γ-^{32}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) Trichloracetic 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 Phosphastase 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:

	Test	Blank
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 ^{3}H 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

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

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 inhibtor 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.