Enzymatic Assay of GLYCOGEN SYNTHASE KINASE 3B

**PRINCIPLE:**

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

Abbreviations used:
- PI-2 = Phosphatase Inhibitor-2
- \(\gamma^{32}\text{P}-\text{ATP}\) = Adenosine 5'-Triphosphate \(\gamma^{32}\text{-P label}\)
- GSK = Glycogen Synthase Kinase 3B
- ADP = Adenosine 5'-Triphosphate

**CONDITIONS:** \(T = 30^\circ\text{C}, \ \text{pH} = 7.5\)

**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. \(\gamma^{32}\text{P}-\text{Adenosine 5'-Triphosphate Solution (}\gamma^{32}\text{-P-ATP)}\)
   (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)

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:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent H (Enzyme Solution)</td>
<td>0.005</td>
</tr>
<tr>
<td>Reagent A (Enz Dil)</td>
<td>------</td>
</tr>
<tr>
<td>Reagent G (PI-2)</td>
<td>0.01</td>
</tr>
<tr>
<td>Reagent C (4x Reaction Buffer)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

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, \( \text{SR} \). \( \text{SR} = \frac{R}{4000} \text{ cpm/pmol.} \)

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 \( C \).
\[
C = (C \text{ sample} - C \text{ blank}) \times \frac{4}{3}
\]

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

\( \text{df} = \) 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 phosphatase 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:


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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.

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