

G-1-P (Potato) Report

Introduction / Theory

Preparation of Enzyme

Use of Phenylmercuric Nitrate

Incubation of Enzyme with Starch

Heat Inactivation

Removal of Inorganic Phosphate, $(\text{Mg}(\text{OAc})_2 \cdot 4 \text{H}_2\text{O})$

How does $\text{Mg}(\text{OAc})_2 \cdot 4 \text{H}_2\text{O}$ remove P_i ??

Decolorization.

Assays- "Fiske-Subbarow"

7-Min Hydrolysis (Indication G-1-P: P_i after Hydrolysis - P_i without Hydrolysis)

How does the hydrolysis work (equation)??

Inorganic Phosphate (P_i)

Determination $\text{P}_i < 15\%$ of 7-Min P

Use of Cation Exchange Resin (Dowex 50)

How does Dowex 50 Work??

Assays- "Fiske-Subbarow"

7-Min Hydrolysis (Indication G-1-P: P_i after Hydrolysis - P_i without Hydrolysis)

Inorganic Phosphate (P_i)

Use of Anion Exchange Resin (Amberlite, IR-45)

How does Amberlite work??

Assays- "Fiske-Subbarow"

7-Min Hydrolysis (Indication G-1-P: P_i after Hydrolysis - P_i without Hydrolysis)

Inorganic Phosphate (P_i)

"Fractions"

Combined Eluent

Graphs:

P_i Standard Curve
Glucose Standard Curve (Nelson's)
Elution Pattern

Tables:

Data
Percent G-1-P Recovered During Purification

Sample Calculations:

Number μ moles P_i and 7-min P- "Aliquots"
Indicate that G-1-P concentration is a comparison of P_i after 7-Min Hydrolysis to
P_i without Hydrolysis
Number μ moles P_i and 7-min P- "Entire Volume"

Results

A Flow chart of the steps in the isolation, indicate the purpose of each step.
Table: Volume, μ moles P_i 7-Min / mL, μ moles P_i 7-Min Total Volume, Percent Yield

Characterization

7-Minute Hydrolysis

Total Hydrolysis

Unhydrolyzed Sample

How many μ moles of P_i contaminate your product??

Results of Nelsons Test (Reducing Equivalent)

How does this assay work??

Where would the G-6-P come from??

Conclusion**Extra "Stuff"**