PAPS Synthesis Kit

Catalog Number: EA005

This kit is designed for the synthesis of 1.25 mCi of [^{35}S]-PAPS. This package insert must be read in its entirety before using this product.

PRINCIPLE FOR PAPS SYNTHESIS

3′-Phosphoadenosine-5′-phosphosulfate (PAPS) is the universal sulfur donor for sulfotransferases. It is synthesized from ATP through two steps. In the first step, one ATP molecule is converted to adenosine-5′-phosphosulfate (APS) by ATP sulfurylase and pyrophosphate (PPi) is generated as a byproduct. In the second step, the APS is converted to PAPS by APS kinase and ADP is generated as a byproduct. To drive the reaction forward, the pyrophosphate can be further degraded to phosphate (Pi) by inorganic pyrophosphatase to eliminate feedback inhibition, and the ADP can be converted back to ATP using pyruvate kinase.

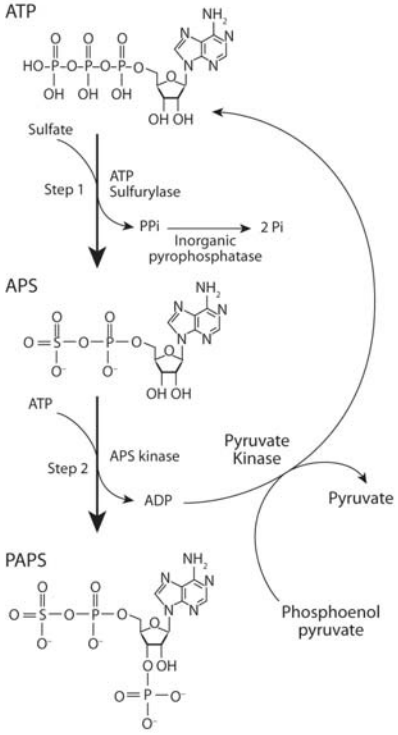

Figure 1: PAPS Synthesis.

MATERIALS PROVIDED

Upon receipt, store all kit components at ≤ -20 °C. Use before the kit expiration date.

PAPS Enzyme Mix (Part 896005, 1 vial) - 25 µL in Tris buffer at pH 7.5 with 25% glycerol. The mix contains ATP sulfurylase, APS kinase, inorganic pyrophosphatase, and pyruvate kinase at the appropriate concentrations for PAPS synthesis.

PAPS Substrate Mix (Part 896004, 1 vial) - 100 µL containing sufficient concentrations of ATP, phosphoenolpyruvate, and enzyme co-factors.

6X Gel Loading Dye (Part 896007, 1 vial) - 1 mL in 20 mM Tris (pH 8.0) with 30% glycerol and 0.02% bromophenol blue.

PAPS Storage Buffer (Part 896006, 1 vial) - 1 mL of a 10X solution of 0.1 M Tris, pH 8.0. Prior to use, dilute to a 1X solution using deionized or distilled water.

MATERIALS REQUIRED

- ^{35}S Sodium Sulfate at 10 mCi/mL or higher specific radioactivity.
- 30 °C incubator.

Optional:

- DEAE-Sepharose column with 0.2 mL bed volume.
- Gel dryer.
- Liquid scintillation counter.
- Phosphorimager or X-ray film and film developer.
- Blotting paper (ThermoFisher, Catalog # 05-714-4 or equivalent).
- Autorad markers (Stratagene, Catalog # 420202 or equivalent).
- SDS-gel electrophoresis apparatus and 8% SDS polyacrylamide gel.

[^{35}S]-PAPS SYNTHESIS PROTOCOL

This protocol is for synthesis of 250 μCi of [^{35}S]-PAPS. The reaction may be scaled up proportionally. For more information, refer to the Technical Hints and Limitations section.

1. Thaw all kit components at room temperature.
2. Combine the ^{35}S Sodium Sulfate, PAPS Substrate Mix, and PAPS Enzyme Mix.

^{35}S Sodium Sulfate	25 μL
PAPS Substrate Mix	20 μL
PAPS Enzyme Mix	5 μL
Total Volume	50 μL

3. Incubate the reaction mix at 30 °C for 16-20 hours.
4. Dilute 2 μL of the reaction mix into 1 mL of deionized or distilled water.
5. Count 10 μL of the diluted sample with a liquid scintillation counter (if available).
6. Based on the counts, dilute the reaction mix to 2 x 10^6 cpm/μL in 1X PAPS Storage Buffer (roughly a 4-fold dilution).
7. Aliquot the preparation, and store at ≤ -20 °C. For tips on long-term storage, see the Technical Hints and Limitations section.

MEASUREMENT OF ^{35}S INCORPORATION (OPTIONAL)

Following [^{35}S]-PAPS synthesis, ^{35}S incorporation may be measured using the following methods.

A. Electrophoresis Separation (see reference 1)

1. Load 2 x 10^6 cpm of prepared [^{35}S]-PAPS on an 8% SDS polyacrylamide gel with one lane open between neighboring samples (load the same amount of free ^{35}S in a separate lane as a control).
2. Run electrophoresis at 10 volts/cm until the dye is halfway in the gel.
3. Place the gel on blotting paper.
4. Dry the gel using a gel dryer.
5. Affix two autorad markers to the sides of the gel.
6. Expose the gel to X-ray film or a phosphoimager for a minimum of 2 hours.
7. Develop the film or phosphoimager plate, and identify the positions of free ^{35}S and [^{35}S]-PAPS (figure 2).
8. Excise the hot spots and count on a liquid scintillation counter to determine incorporation.

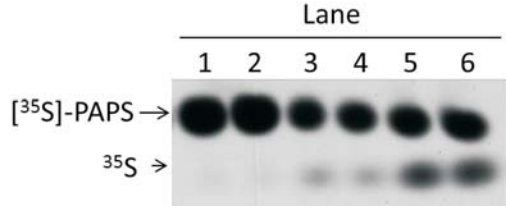

Figure 2. [^{35}S]-PAPS samples separated on an 8% SDS polyacrylamide gel.

Lanes 1 and 2 (freshly prepared [^{35}S]-PAPS): All the ^{35}S is incorporated into [^{35}S]-PAPS.

Lanes 3 and 4 ([^{35}S]-PAPS that has been stored in 1X PAPS Storage Buffer with 50% ethanol for 6 months): 4% of [^{35}S]-PAPS is degraded into PAP and free ^{35}S.

Lanes 5 and 6 ([^{35}S]-PAPS that has been stored in 1X PAPS Storage Buffer without ethanol for 6 months): 15% of [^{35}S]-PAPS is degraded.

B. Chromatography Separation

1. Load 2 x 10^6 cpm of prepared [^{35}S]-PAPS onto a DEAE-Sepharose column with a 0.2 mL bed volume.
2. Wash the column with 1 mL of 25 mM Tris (pH 7.5) and 40 mM NaCl to remove free ^{35}S.
3. Elute [^{35}S]-PAPS with 1 mL of 25 mM Tris (pH 7.5) and 150 mM NaCl.
4. To determine incorporation, count both the wash and the eluate with a liquid scintillation counter.

TECHNICAL HINTS AND LIMITATIONS

- This kit is designed for [^{35}S]-PAPS synthesis but may be used for non-radioactive PAPS synthesis. In the case of non-radioactive PAPS synthesis, 0.1 M Sodium Sulfate is recommended for the sulfate input and 0.1 μmol PAPS may be synthesized.
- The optimal pH range for synthesis is 7.0 to 8.0. In the case where the input sulfate will significantly shift the pH, an adjustment to this pH range is recommended.
- During storage, degradation of PAPS will occur slowly. After 1 half-life storage (87 days), it is expected that less than 10% of PAPS is degraded. 3′-Phosphoadenosine-5′-phosphate (PAP) and free sulfate are the major degradation products. A minor portion of PAPS may also be degraded into APS and free phosphate under certain conditions (2). During gel electrophoresis, ^{35}S moves faster than [^{35}S]-PAPS and [^{35}S]-APS moves slower than [^{35}S]-PAPS.
- Storage in 50% ethanol may greatly improve the stability of PAPS. For storage in 50% ethanol, add an equal volume of 100% ultrapure ethanol into the PAPS preparation.
- No purification of [^{35}S]-PAPS is necessary. The preparation can be used directly in a sulfotransferase assay or labeling.
- If purification of PAPS is required, it may be purified using anion exchange chromatography. PAPS has a higher retention time on DEAE resin than the contaminating free sulfate, AMP, ADP, and ATP and can be eluted with 25 mM Tris (pH 7.5) and 150 mM NaCl.
- The concentration of pure PAPS can be determined by measuring absorbance at 260 nm (extinction coefficient of 15,400 $M^{-1}cm^{-1}$).

REFERENCES

1. Wu, Z.L. *et al.* (2010) BMC Biotechnology **10**:11.
2. Prather, B. *et al.* (2012) Anal. Biochem. **423**:86.

NOTES

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