CREATIVE BIOMART®
Assay Kit

PAPS Synthesis Kit

Product Information

Common Name

PAPS

Cat.No. Kit-2171

Description

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.

Storage

Upon receipt, store all kit components at -20 °C.

Synonyms

3-Phosphoadenosine-5-phosphosulfate; PAPS; 3-Phosphoadenosine-5-phosphosulfate Synthesis Kit

Kit Components

PAPS Enzyme Mix (1 vial) - 25 uL 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 (1 vial) - 100 uL containing sufficient concentrations of ATP, phosphoenolpyruvate, and enzyme co-factors.
6X Gel Loading Dye (1 vial) - 1 mL in 20 mM Tris (pH 8.0) with 30% glycerol and 0.02% bromophenol blue.
PAPS Storage Buffer (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 but Not Supplied

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

Technical Notes

1. This kit is designed for [35S]-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 mol PAPS may be synthesized.
2. 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.
3. 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, 35S moves faster than [35S]-PAPS and [35S]-APS moves slower than [35S]-PAPS.
4. 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.
5. No purification of [35S]-PAPS is necessary. The preparation can be used directly in a sulfotransferase assay or labeling.
6. 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.
7. The concentration of pure PAPS can be determined by measuring absorbance at 260 nm (extinction coefficient of 15,400 M-1cm-1).

Assay Protocol

[35S]-PAPS SYNTHESIS PROTOCOL
1. Thaw all kit components at room temperature.
2. Combine the 35S Sodium Sulfate, PAPS Substrate Mix, and PAPS Enzyme Mix.
35S Sodium Sulfate: 25 L
PAPS Substrate Mix: 20 L
PAPS Enzyme Mix: 5 L

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Total Volume: 50 L
3. Incubate the reaction mix at 30 °C for 16-20 hours.
4. Dilute 2 L of the reaction mix into 1 mL of deionized or distilled water.
5. Count 10 L 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/L 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 35S INCORPORATION
A. Electrophoresis Separation
1. Load 2 x 106 cpm of prepared [35S]-PAPS on an 8% SDS polyacrylamide gel with one lane open between neighboring samples (load the same amount of free 35S 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 35S and [35S]-PAPS.
8. Excise the hot spots and count on a liquid scintillation counter to determine incorporation.
B. Chromatography Separation
1. Load 2 x 106 cpm of prepared [35S]-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 35S.
3. Elute [35S]-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.

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