**DESCRIPTION**

**Source**  
E. coli-derived yeast ATP-Sulfurylase/MET3 protein  
Pro2-Phe511, with N-terminal Met and 6-His tag  
Accession # P08536

**N-terminal Sequence Analysis**  
Met

**Predicted Molecular Mass**  
59 kDa

**SPECIFICATIONS**

**SDS-PAGE**  
57-58 kDa, reducing conditions

**Activity**  
Measured by its ability to sulfurylate ATP.  
The specific activity is >2,500 pmol/min/µg, as measured under the described conditions.

**Endotoxin Level**  
<1.0 EU per 1 µg of the protein by the LAL method.

**Purity**  
>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

**Formulation**  
Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol and DTT. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**

- Assay Buffer: 25 mM Tris, 1 mM MgCl₂, 20 mM Na₂SO₄, pH 8.0  
- Recombinant Yeast ATP Sulfurylase/MET3 (ryMET3) (Catalog # 7175-AS)  
- Recombinant Human Inorganic Pyrophosphatase/PPA1 (rhPPA1) (Catalog # 6557-PP)  
- Recombinant *Penicillium chrysogenum* APS Kinase/APSK (rAPS Kinase) (Catalog # 7176-SK)  
- Adenosine Triphosphate (ATP) (Sigma, Catalog # A7699), 10 mM stock in deionized water  
- Malachite Green Phosphate Detection Kit (Catalog # DY996)  
- 96-well Clear Plate (Costar, Catalog # 92592)  
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

**Assay**

1. Dilute ATP to 1.8 mM in Assay Buffer.
2. Dilute rhPPA1 to 3 µg/mL in Assay Buffer.
3. Dilute rAPS Kinase to 120 µg/mL in Assay Buffer.
4. Prepare Reaction Mixture by combining equal volumes of 1.8 mM ATP, 3 µg/mL rhPPA1, and 120 µg/mL rAPS Kinase.
5. Dilute ryMET3 to 0.2 µg/mL in Assay Buffer.
6. Dilute 1 M Phosphate Standard by adding 10 µL of the 1 M Phosphate Standard to 990 µL of deionized water for a 10 mM stock.  
   Continue by adding 10 µL of the 10 mM Phosphate stock to 990 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
7. Continue standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer.  
   The standard curve has a range of 0.078 to 5.0 nmol per well.
8. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
9. Load 25 µL of the 0.2 µg/mL ryMET3 into the plate. Include a Control containing 25 µL of Assay Buffer.
10. Start the reaction by adding 25 µL of Reaction Mixture to the wells, excluding the standard curve and curve blank.
11. Cover the plate with parafilm or a plate sealer and incubate at 37 °C for 20 minutes.
12. Add 30 µL of the Malachite Green Reagent A to all wells.
13. Add 100 µL of deionized water to all wells. Mix briefly.
14. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
15. Read plate at 620 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

   \[
   \text{Specific Activity (pmol/min/µg)} = \left( \frac{\text{Phosphate released* (nmol)}}{1000 \text{ pmol/nmol}} \right) \times \frac{1}{\text{Incubation time (min)} \times \text{amount of enzyme (µg)}} + 2**
   \]

   *Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.  
   **Two inorganic phosphates are generated from each MET3 produced pyrophosphate

**Final Assay Conditions**  
Per Reaction:  
- ryMET3: 0.005 µg  
- ATP: 0.3 mM  
- rhPPA1: 0.025 µg  
- rAPS Kinase: 1 µg

**PREPARATION AND STORAGE**

**Shipping**  
The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage**  
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.  
- 6 months from date of receipt, -70 °C as supplied.  
- 3 months, -70 °C under sterile conditions after opening.
Sulfur metabolism is essential to all organisms. Inorganic sulfate is sequentially activated by ATP Sulfurylase and adenosine 5'-phosphosulfate kinase (APSK). ATP Sulfurylase converts ATP to adenosine-5'-phosphosulfate (APS) and generates pyrophosphate (1). APSK subsequently phosphorylates APS at the 3' site to generate 3'-phosphoadenosine-5'-phosphosulfate (PAPS) (2). PAPS is the sulfur donor required for all sulfotransferase reactions in humans and is also the sulfur source for synthesis of cysteine, methionine and glutathione (3). The ATP Sulfurylase reaction is highly unfavorable energetically with the standard free-energy change of approximately +46 kJ/mol (4). To drive the reaction forward, APSK and pyrophosphatase are generally added into the reaction simultaneously to remove the products. The recombinant S. cerevisiae ATP Sulfurylase can be used for in vitro PAPS synthesis (5, 6).

References: