Recombinant Human MAT2A His-tag

Catalog Number: 10315-MA

DESCRIPTION

Source	*E. coli*-derived human MAT2A protein Asn2-Tyr395 Accession # P31153-1 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	44 kDa

SPECIFICATIONS

SDS-PAGE	48-55 kDa, under reducing conditions
Activity	Measured via hydrolysis of ATP during synthesis of S-adenosylmethionine. The specific activity is >85 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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, TCEP and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 50 mM KCl, 10 mM $MgCl_2$, 0.05% Brij-35, pH 8.0
- Recombinant Human Methionine Adenosyltransferase 2A (rhMAT2A) (Catalog # 10315-MA)
- L-Methionine (Sigma, Catalog M5308), 300 mM stock in deionized water
- ATP (Sigma, Catalog # A7699), 400 mM stock in deionized water
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 M Phosphate Standard provided by the Malachite Green Phosphate Detection Kit by adding 10 µL of the 1 M Phosphate Standard to 990 µL of Assay Buffer 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.
2. 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 nmoles per well.
3. Prepare Reaction Mixture containing 400 µM ATP and 600 µM L-Methionine in Assay Buffer.
4. Dilute rhMAT2A to 20 µg/mL in Assay Buffer.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
6. Load 25 µL of the 20 µg/mL rhMAT2A into empty wells of the plate. Include a Control containing 25 µL of Assay Buffer.
7. Start the reactions by adding 25 µL of Reaction Mixture to all the wells, excluding the standard curve and curve blank.
8. Seal the plate and incubate at room temperature for 20 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rhMAT2A: 0.5 µg
- ATP: 200 µM
- L-Methionine: 300 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. 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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

S-adenosylmethionine synthase 2 (MAT2A), also known as methionine adenosyltransferase 2, is a member of the AdoMet synthase family, essential magnesium- and potassium-dependent enzymes that catalyze the formation of S-adenosylmethionine (SAMe) which is required for methylation of nucleic acids, phospholipids, histones, biologic amines and proteins (1). MAT2A, the primarily extrahepatic-expressed 395 amino acid isoform has 84% sequence identity to the MAT1A isoform which is exclusively expressed in the liver (1). MAT2A is found in the cytosol and also in the nucleus where it can function as a transcriptional regulator (2, 3). MAT2A primarily exists in active dimeric conformation composed of two monomeric subunits that contribute residues to form two active sites at the subunit interface (4). The MAT2A structure contains a gating loop, common to both alpha isoforms, that regulates access to the active site (4). Association of MAT2A with a regulatory MAT2B subunit can lead to formation of a tetramer with increased function and stability (5, 6). MAT proteins play important roles in chronic liver disease and a switch in their expression patterns is observed in liver cancers (7). MAT2A is induced in hepatocarcinogenesis as a marker of rapid liver growth (1, 8, 9). Lower SAMe levels resulting from the MAT isoform switch results in proliferative signaling in the liver (10). In extrahepatic cancer cells, increased MAT2A supplies SAMe for polyamine biosynthesis and can act as a transcription factor for BCL-2 as well as physically interacting to promote stability (3). MAT2A is elevated in several cancers including colon (11), gastric (12), pancreatic (6), and prostate (6) leading to enhancement of tumor migration and invasion (6). MAT2A inhibition is a marker and proposed target for cancer therapy (6, 11-14).

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