Recombinant Human MTAP His-tag

Catalog Number: 10379-MT

DESCRIPTION

Source	*E. coli*-derived human MTAP protein Ala2-His283 Accession # Q13126-1 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	32 kDa

SPECIFICATIONS

SDS-PAGE	30-32 kDa under reducing conditions
Activity	Measured by its ability to produce adenine through the conversion of 5'methylthioadenine to 5'methylthioribose-1-phosphate. The specific activity is >650 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 and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Potassium Phosphate, 1 mM DTT, pH 7.4
- Recombinant Human Methylthioadenosine Phosphorylase (rhMTAP) (Catalog # 10379-MT)
- 5'-Deoxy-5'-Methylthioadenosine (MTA) (Cayman Chemical, Catalog # 15593), 30 mM stock in DMSO
- Xanthine Oxidase (Sigma, Catalog # X2252), 200 U/mL stock in 10 mM Potassium Phosphate, pH 7.4
- UV Plate (Costar, Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhMTAP to 2 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 600 µM MTA and 6 U/mL Xanthine Oxidase in Assay Buffer.
3. Load into a plate 50 µL of 2 µg/mL rhMTAP, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate Mixture.
4. Seal the plate and incubate at room temperature for 15 minutes.
5. Read plate at 305 nm (absorbance) in kinetic mode for 5 minutes.
6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using extinction coefficient 15500 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhMTAP: 0.1 µg
- MTA: 300 µM
- Xanthine Oxidase: 3 U/mL

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PREPARATION AND STORAGE

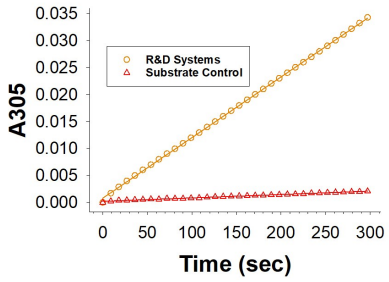
Shipping The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

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.

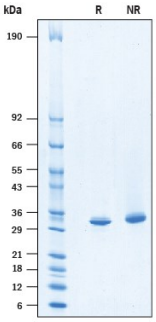
DATA

Enzyme Activity



Recombinant Human MTAP His-tag (Catalog # 10379-MT) is measured by its ability to produce adenine through the conversion of 5'methylthioadenine to 5'methylthioribose-1 phosphate.

SDS-PAGE



2 µg/lane of Recombinant Human MTAP His-tag (Catalog # 10379-MT) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 30-32 kDa under reducing conditions.

BACKGROUND

Methyl-thioadenosine phosphorylase/MTAP is part of the PNP/MTAP phosphorylase family and catalyzes the reversible phosphorylation of S-methyl-5'-thioadenosine (MTA), a major byproduct of polyamine synthesis essential for cell growth and proliferation. MTAP also produces most of the free adenine generated in human cells through a salvage pathway and thus couples the purine salvage pathway with polyamine biosynthesis. MTAP forms an active trimer where each identical 32 kDa monomer contains a separate active site (1). Each active site contains three distinct regions required for base-, methylthioribose-, and sulfate/phosphate-binding (1). MTAP is cytosolic and abundantly expressed in normal cells and tissues (2). In contrast, deficient MTAP expression is observed in many types of tumors including lung, bladder, pancreatic, and endometrial cancer (3) due to hyper-methylation gene suppression (4) or gene deletion (3, 5, 6). MTA accumulation leads to an immunosuppressive tumor microenvironment and apoptotic resistance (7-9) and MTAP directly regulates the level of MTA present. MTAP has been reported as a tumor suppressor (6,10) that may also act in a manner that is independent of enzymatic activity (11) through signaling pathways such as the insulin-like growth factor-1 receptor pathway (12). Potential therapeutic strategies to exploit MTAP deficiency in tumors (13,14) or inhibit MTAP in tumors that express MTAP, such as prostate cancer, are under investigation (15).

References:

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