Recombinant Human IMP Dehydrogenase 1/IMPDH1

Catalog Number: 8904-DH

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Glu2-Tyr563, with a N-terminal Met and 6-His tag Accession # NP_899066
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	61 kDa

SPECIFICATIONS

SDS-PAGE	53-64 kDa, reducing conditions
Activity	Measured by its ability to convert the substrate inosine-5'-phosphate (IMP) to xanthosine-5'-phosphate (XMP). The specific activity is >100 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	>90%, 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, EDTA, Glycerol and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 300 mM NaCl, pH 8.0
- Recombinant Human IMP Dehydrogenase 1/IMPDH1 (rhIMPDH1) (Catalog # 8904-DH)
- Nicotinamide adenine dinucleotide sodium salt (β-NAD) (Sigma, Catalog # N6522), 100 mM stock in deionized water
- Inosine 5'-monophosphate (IMP) (Sigma, Catalog # I4625), 100 mM stock in deionized water
- UV plate (Costar, Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhIMPDH1 to 20 µg/mL in Assay Buffer.
2. Create Substrate Mixture containing 200 µM IMP and 400 µM β-NAD in Assay Buffer.
3. Load 50 µL of 20 µg/mL rhIMPDH1 into a plate, and start the reaction by adding 50 µL of Substrate Mixture. For Substrate Blank, load 50 µL of Assay Buffer and 50 µL of Substrate Mixture.
4. Read plate at a wavelength of 340 nm (bottom read) in kinetic mode for 5 minutes.
5. 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 the extinction coefficient 6220 $M^{-1}cm^{-1}$
***Using the path correction 0.320 cm
Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhIMPDH1: 1.0 µg
- β-NAD: 200 µM
- IMP: 100 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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USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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BACKGROUND

IMPDH1 (inosine monophosphate dehydrogenase) is a ubiquitous cytosolic and nuclear enzyme that plays a central role in guanine nucleotide metabolism. It catalyzes the NAD-dependent conversion of inosine monophosphate (IMP) to hypoxanthine monophosphate (XMP) which is a precursor for the synthesis of GMP, guanosine, and guanine. These compounds are critical for DNA synthesis and cell proliferation (1, 2). IMPDH1 associates into a homotetramer of approximately 55 kDa subunits, and the tetramers can aggregate into perinuclear structures (3, 4). IMPDH1 binds to the nucleotides AMP, ATP, IMP, and XMP (4) as well as to single stranded DNA and RNA (5). It is inhibited by the immunosuppressant drug mycophenolic acid (MPA) (3, 6) which induces reversible IMPDH1 aggregation (7). It is required for vascular endothelial cell proliferation and the terminal differentiation of adipocytes (8, 9). Mutations of IMPDH1 are associated with autosomal dominant retinitis pigmentosa (4, 10). Human IMPDH1 shares 98% amino acid sequence identity with mouse and rat IMPDH1. Alternative splicing generates additional isoforms with internal deletions or variable substitutions at the N-terminus.

References:

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