Recombinant Human Inosine 5'-Monophosphate Dehydrogenase 2/IMPDH2

Catalog Number: 8349-DH

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human IMP Dehydrogenase 2/IMPDH2 protein Met1-Phe514, with a C-terminal 10-His tag Accession # P12268
N-terminal Sequence Analysis	Met1 predicted
Predicted Molecular Mass	57 kDa

SPECIFICATIONS

SDS-PAGE	52-61 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 >30 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	>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, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 300 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 8.0
- Recombinant Human Inosine 5'-Monophosphate Dehydrogenase 2/IMPDH2 (rhIMPDH2) (Catalog # 8349-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 rhIMPDH2 to 20 µg/mL in Assay Buffer.
2. Create Substrate Mixture containing 500 µM IMP and 1 mM β-NAD in Assay Buffer.
3. Load 50 µL of 20 µg/mL rhIMPDH-2 into a plate, and start the reaction by adding 50 µL of Substrate Mixture. For Substrate Blanks, load 50 µL of Assay Buffer and 50 µL of Substrate Mixture.
4. Read plate at a wavelength of 339 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:

- rhIMPDH-2: 1.0 µg
- β-NAD: 500 µM
- IMP: 250 µM

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, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

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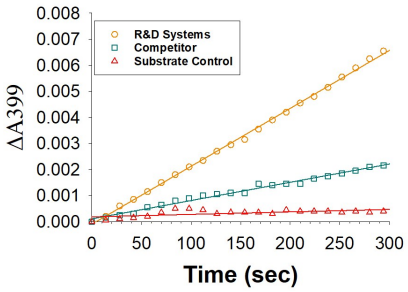
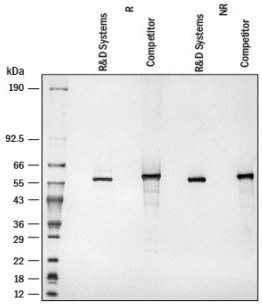
DATA

Enzyme Activity



Recombinant Human IMPDH2 (Catalog # 8349-DH) is measured by its ability to convert the substrate inosine-5'-phosphate (IMP) to xanthosine-5'-phosphate (XMP). The activity (orange) is approximately 3.5-fold greater than the competitor's IMPDH2 (green).

SDS-PAGE



1 µg/lane of Recombinant Human Inosine 5'-Monophosphate Dehydrogenase 2/IMPDH2 was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silverstaining, showing a band at 57 kDa.

BACKGROUND

IMPDH2 (inosine monophosphate dehydrogenase) is one of two cytosolic and nuclear enzyme isoforms that play a central role in guanine nucleotide metabolism. The isoforms are 84% identical but distinctly regulated. While IMPDH1 is generally constitutively expressed, IMPDH2 is inducible during proliferation and transformation (1, 2). Both isoforms form a homotetramer of approximately 55 kDa monomers containing a catalytic barrel domain and a subdomain with two cystathione beta-synthase domains which mediate RNA and DNA binding (1, 3). Both enzymes catalyze 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 (4, 5) which explains the importance of IMPDH in cancer and viral infection (6-9). Although IMPDH1 and IMPDH2 are known to be inhibited by the immunosuppressant drug mycophenolic acid (MPA), much research has targeted discovery of additional and selective inhibitors for IMPDH isoforms given they are targets for several major therapeutic areas (1, 9-13). Human IMPDH2 shares 99% amino acid sequence identity with mouse IMPDH2.

References:

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