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Human Inosine-5'-Monophosphate Dehydrogenase (IMPDH) Inhibitor Screening Assay Kit *09/17*

(Catalog # K495-100; 100 assays, Store kit at -20°C)

I. Introduction:

Inosine monophosphate dehydrogenase, IMPDH, (E.C. 1.1.1.205) is the rate-limiting enzyme in de novo guanine nucleotide biosynthesis and it is essential for lymphocyte proliferation. IMPDH oxidizes inosine 5'-monophosphate (IMP) to xanthine 5'-monophosphate (XMP) using NAD as a cofactor. It plays a critical role in cell growth and in the malignancy of some tumors such as pancreatic, colon and bladder cancers. IMPDH2 is the predominant isoform of IMPDH and is specifically linked to a wide range of cancers and lymphocyte proliferation. IMPDH is recognized as a validated target as antiviral, antiparasitic, antimicrobial, antileukemic and immunosuppressive agents. In BioVison's IMPDH inhibitor screening assay, IMP is oxidized by IMPDH producing a series of intermediates, which react with the probe to generate a colorimetric signal (OD 450 nm). The signal is directly proportional to the IMPDH activity and has a very low background. In the presence of a IMPDH inhibitor such as mycophenolic acid (MPA), the reaction is arrested, thus decreasing the signal. BioVision's IMPDH assay is fast, sensitive and reproducible and is suitable for functional assays, high-throughput screening and preclinical studies in drug discovery.

$$\text{IMP + Cofactor} \xrightarrow{\text{IMPDH + Inhibitor}} \text{XMP + Intermediate} \xrightarrow{\text{Detection Solution}} \text{Color (OD 450 nm)}$$

II. Application:

- High-throughput screening, drug discovery or characterization of IMPDH2 inhibitors

III. Kit Contents:

Components	K495-100	Cap Code	Part Number
Assay Buffer	25 ml	WM	K495-100-1
IMPDH Substrate	1 vial	Blue	K495-100-2
IMPDH Detection	1 vial	Red	K495-100-3
IMPDH2 Enzyme	200 µl	Green	K495-100-4
MPA (10 mM)	20 µl	Purple	K495-100-5

IV. User Supplied Reagents and Equipment:

- 96-well clear flat-bottom plate
- Multi-well spectrophotometer

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **Assay Buffer**: Ready to use as supplied. Bring it to room temperature before use. Store at 4°C.
- **IMPDH Substrate**: Reconstitute with 1.2 ml of Assay Buffer. Keep on ice after use. Store at -20°C.
- **IMPDH Detection**: Reconstitute with 220 µl of Assay Buffer. Keep on ice after use. Store at -20°C.
- **IMPDH2 Enzyme**: Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze thaw.
- **MPA (10 mM)**: Ready to use as supplied. Bring it to room temperature before use. Store at -20°C.

VI. Human Inosine-5'-Monophosphate Dehydrogenase II Inhibitor Screening Assay Protocol:

1. **Screen Compounds, Inhibitor Control and Enzyme Activity Control Preparations:** Make a stock solution of the candidate compounds in appropriate solvent(s) (e.g. DMSO). If desired, prepare serial dilutions of your testing compounds appropriately. *High solvent concentration might affect the enzymatic activity.* Thus, prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzymatic activity (10 µl; 20% DMSO/Assay Buffer). Make working solutions of the candidate compounds by diluting the stock solutions to 20X the test concentration in Assay Buffer just before use. For all inhibitor candidates and solvent controls, solvent concentration should not be greater than 1% final concentration. For Inhibitor Control (MPA): dilute the stock solution (1:10) in Assay Buffer to prepare a working solution. Add 10 µl of the candidate compound working solutions [S], Inhibitor Control (MPA) working solution [IC], Solvent Controls [SC] or Assay Buffer [enzyme control; EC] into appropriate wells.
2. **Enzyme Solution Preparation**: Mix enough reagents for the number of assays to be performed. For each assay, prepare 2-fold dilution of IMPDH Enzyme by using assay buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Add 178 µl of Reaction Mix to the wells containing 10 µl of candidate solutions, inhibitor control, solvent control or enzyme control. *The volume now is 188 µl/well.*

	Reaction Mix
Assay Buffer	174 µl
Diluted Enzyme	4 µl

4. **Inhibitor Incubation:** Incubate the inhibitor or compound with the enzyme for 30 min. at room temperature.
5. **Substrate Mix:** Mix enough reagents for the total number of samples to be assayed. For each well, prepare 12 µl of Substrate Mix containing 10 µl of IMPDH Substrate and 2 µl of IMPDH Detection. Mix well. Add 12 µl of Substrate Mix per well Mix well. *The final reaction volume is 200 µl/well*.

6. **Measurement:** Measure the OD at 450 nm at 37°C for 60 min in kinetic mode. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding OD values for the fluorescence (OD_1 and OD_2).
7. **Calculations:** Calculate the slope for all samples, including Enzyme Activity Control [EC], and Solvent Control [SC], by dividing the net ΔOD ($OD_2 - OD_1$) values with the time Δt ($t_2 - t_1$). Calculate % Relative Inhibition as follows (in case SC values are significantly different from EC values use the SC values in the equations below):

$$\textbf{Inhibition } (\%) = \frac{[EC] - [S]}{[EC]} \times 100\%$$

$$\textbf{Relative Activity } (\%) = \frac{[S]}{[EC]} \times 100\%$$

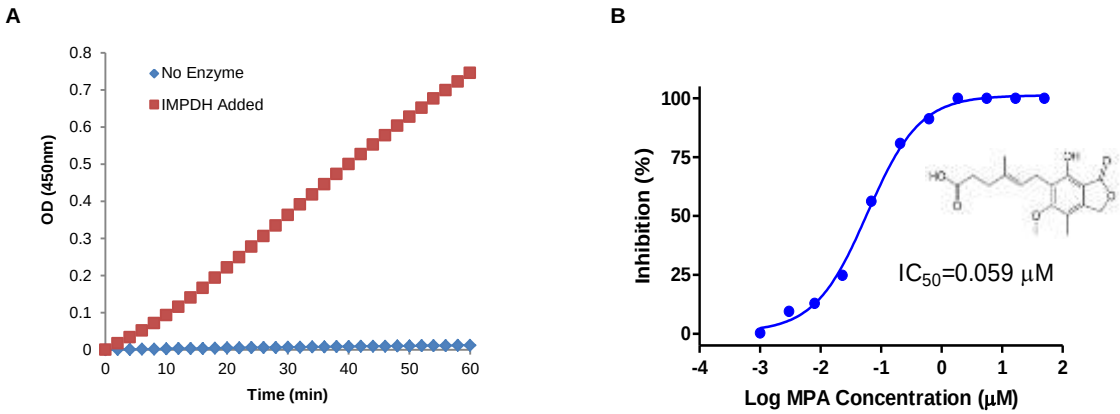

Figure A shows the reaction kinetics in the presence and absence of the IMPDH2 enzyme. **B.** Inhibition of IMPDH2 enzymatic activity using MPA (IC_{50}: 59 nM).

VII. RELATED PRODUCTS:

Inosine Fluorometric Assay Kit (K712)
Xanthine Oxidase Activity Colorimetric/Fluorometric Assay Kit (K710)
Xanthine/Hypoxanthine Colorimetric/Fluorometric Assay Kit (K685)
Adenosine Deaminase Activity Assay Kit (Fluorometric) (K328)
Adenosine Deaminase Activity Assay Kit (Colorimetric) (K768)
Purine Nucleoside Phosphorylase Activity Assay Kit (Fluorometric) (K767)
Purine Nucleoside Phosphorylase Activity Assay Kit (Colorimetric) (K768)
Human Inosine-5′-Monophosphate Dehydrogenase II, human recombinant (P1131-20)
HPRT1, human recombinant (Cat. no. P1092-50)
2'-Deoxy-6-thioguanosine (Cat. no. 2828-25)

FOR RESEARCH USE ONLY! Not to be used on humans.