ab283395 – IMPDH2 Inhibitor Screening Kit (Fluorometric)

For the screening of potential IMPDH2 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283395

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. The kit components are stable for one year when stored as recommended.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer	25 mL	-20°C
IMPDH Substrate	1 vial	-20°C
IMPDH Detection Mix	1 vial	-20°C
IMPDH2 Enzyme	200 µL	-20°C
MPA (10 mM)	20 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Briefly centrifuge small vials prior to opening.

Assay Buffer: Ready to use as supplied. Warm to room temperature (RT) 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.

Assay Protocol

Screen Compounds, Inhibitor Control and Enzyme Activity Control Preparations:

1. Dissolve the test compound in appropriate solvent.
2. If desired, prepare serial dilutions of your testing compounds appropriately.
3. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzymatic activity (10 µL; 20% DMSO/Assay Buffer).
4. Make working solutions of the candidate compounds by diluting the stock solutions to 20X the test concentration in Assay Buffer just before use.
5. For all inhibitor candidates and solvent controls, solvent concentration should not be greater than 1% final concentration.
6. For Inhibitor Control (MPA): dilute the stock solution (1:10) in Assay Buffer to prepare a working solution.
7. 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.

Δ Note: *High solvent concentration might affect the enzymatic activity.*

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.

Reaction Mix:

1. Mix enough reagents for the number of assays to be performed.
2. 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

Inhibitor Incubation: Incubate the inhibitor or compound with the enzyme for 30 minutes at room temperature.

Substrate Mix:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 12 µL of Substrate Mix containing 10 µL of IMPDH Substrate and 2 µL of IMPDH Detection.
2. Mix well. Add 12 µL of Substrate Mix per well, mix well. The final reaction volume is 200 µL/well.

Measurement

Measure the OD at 450 nm at 37°C for 60 minutes 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).

Calculation:

1. 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):

$$\% \text{ Inhibition} = \frac{[EC]-[S]}{[EC]} \times 100\%$$

$$\% \text{ Relative activity} = \frac{[S]}{[EC]} \times 100\%$$

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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