ab283388 – Monoacylglycerol Lipase/MGL Screening Kit (Fluorometric)

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

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283388

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
MAGL Assay Buffer	25 mL	-20°C
MAGL Substrate	50 µL	-20°C
Human Monoacylglycerol Lipase	1 vial	-20°C
MAGL Inhibitor	100 µL	-20°C

PLEASE NOTE: Human Monoacylglycerol Lipase was previously labelled as MGL Enzyme, and MAGL Assay Buffer as MGL Assay Buffer. MAGL Inhibitor was previously labelled as MGL Control Inhibitor (JJKK-048). The composition has not changed.

Materials Required, Not Supplied

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

- Black 96-well plate with flat bottom
- Multi-well spectrophotometer
- Anhydrous (reagent-grade) DMSO
- Test Compounds

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

MAGL Assay Buffer: Warm to room temperature before use. Store at -20°C. Use within two months.

MAGL Substrate: Provided as a 200X stock solution in DMSO. Prior to use, warm to room temperature. Aliquot and store at -20°C. Avoid repeated freeze thaw cycles and protect from light. Use within two months.

Human Monoacylglycerol Lipase: Reconstitute with 220 µL MAGL Assay Buffer to prepare a 50X stock solution. Aliquot and store at -20°C. Avoid repeated freeze thaw cycles and use within two months.

MAGL Inhibitor: Provided as a 2 mM stock solution in DMSO. Warm to room temperature before use. Aliquot and store at -20°C. Use within two months.

Assay Protocol

Human Monoacylglycerol Lipase Preparation:

Mix enough reagents for the number of assays to be performed (remember to account for any control reactions such as no inhibitor/solvent control and positive inhibition control wells when calculating the amount of Human Monoacylglycerol Lipase

1. Solution to prepare).
2. For each well, prepare a total of 90 µL Human Monoacylglycerol Lipase, consisting of:

	Preparation Mix
MAGL Assay Buffer	88 µL
Human Monoacylglycerol Lipase	2 µL

3. Add 90 µL of the Human Monoacylglycerol Lipase to each reaction well. Also prepare a background control (no enzyme) well by adding 95 µL MAGL Assay Buffer to an empty well.

Test Compound, Positive Inhibition Control & No Inhibitor Control Preparations:

1. Dissolve test compounds for screening into appropriate solvents to generate stock solutions.
2. For each test compound, prepare a working solution that is 20X the desired test concentration by diluting the stock solution with MAGL Assay Buffer (or desired solvent if compound solubility is a concern).
3. To determine IC_{50} values for test compounds, 20X solutions should be prepared in a range of concentrations in order to generate a multi-point dose-response curve (the amount of organic solvent should be the same for all test concentrations).

Δ Note: *DMSO has no appreciable effect on the activity of MGL, even at concentrations as high as 10% (v/v). If solvents other than DMSO are used to make test compound stock solutions, we recommend preparing a solvent control well with the same final concentration of solvent used to solubilize test compounds and using this well to define 100% activity if different from no inhibitor control well(s).*

4. Prepare reaction wells containing test compounds, as well as corresponding no inhibitor control (which may also serve as a solvent control (SC), if desired) and positive inhibition control wells.
5. Add 5 µL of each 20X test compound solution to test compound wells. For no inhibitor control wells, add 5 µL of MAGL Assay Buffer.
6. A positive inhibition control well may also be prepared using the MGL Control Inhibitor.
7. Dilute the stock at a 1:10 ratio by adding 5 µL of the 2 mM solution to 45 µL MAGL Assay Buffer, yielding a 200 µM working solution (20X final concentration) and add 5 µL of the 20X solution to each positive inhibition control well.
8. Preincubate the plate for 30 min at 37°C (protected from light), to allow test compounds to interact with MGL.

Substrate Mix:

1. During the preincubation period, prepare a 20X working solution of MAGL Substrate by diluting the 200X stock at a 1:10 ratio with anhydrous DMSO.
2. Add 5 µL of the MAGL Substrate working solution (20X) to each reaction well, including background control (no enzyme) well(s).

Δ Note: *The 20X working solution should be aliquoted and store at -20°C.*

Measurement:
Measure the fluorescence at Ex/Em = 360/460 nm in kinetic mode for 30-60 minutes at 37°C. While the assay can be performed in either endpoint or kinetic mode, it is recommended to read in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction.

Calculation:

1. For each reaction well (including no inhibitor and background control wells), choose two time points (T_1 and T_2) in the linear range of the reaction progress curve (excluding the first five minutes of the assay) and obtain the corresponding values for the fluorescence at those times (RFU_1 and RFU_2).
2. Determine the change in fluorescence over the time interval for all reaction wells: $\Delta F/\Delta T = (RFU_2 – RFU_1) / (T_2 – T_1)$.
3. Subtract the rate of the background control well from the rates of each of the no inhibitor, test compound and positive inhibition control wells to determine background-corrected reaction rates.
4. Relative activity can be calculated as follows:

$$\% \text{ Inhibition} = \frac{Rate\ of\ No\ inhibitor\ control - rate\ of\ \text{test compound}}{Rate\ of\ No\ inhibitor\ control} \times 100$$

$$\% \text{ Relative activity} = \frac{Rate\ of\ test\ compound}{Rate\ of\ No\ inhibitor\ control} \times 100$$

Technical Support

For all technical or commercial enquiries please go to:

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