Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

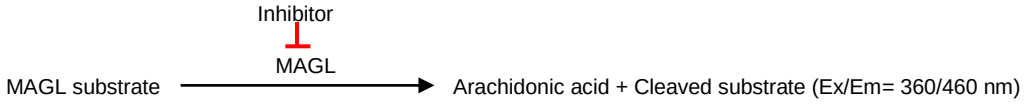
Monoacylglycerol Lipase Inhibitor Screening Kit (Fluorometric)

12/17

(Catalog # K474-100; 100 assays; Store at -20°C)

I. Introduction:

Monoacylglycerol Lipase (**MAGL**, E.C. **3.1.1.23**) is an important enzyme that regulates endocannabinoid signaling in human physiology. MAGL is a serine hydrolase that generates free fatty acid and glycerol from monoacylglycerol stores. One free fatty acid that is frequently generated by MAGL activity, arachidonic acid, is a precursor for eicosanoids, a family of pro-inflammatory signaling molecules. The glycerol moiety can also serve various purposes as a metabolic building block and energy source. Inhibition or inactivation of MAGL leads to 2-arachidonalglycerol (2-AG) buildup, and this has myriad biological consequences. Desensitization of the endocannabinoid receptors and reduced addiction withdrawal symptoms are one outcome of MAGL inhibition. In addition, the downstream eicosanoid signaling pathway is affected; this pathway plays a role in diverse physiological processes including inflammation, immunity, nociception and blood pressure. As a central node in the regulation of both lipid signaling and energy mobilization, MAGL is of considerable interest as a therapeutic target. BioVision's Monoacylglycerol Lipase Inhibitor Screening Kit provides a straightforward method to identify inhibitors of human MAGL. In the assay, human recombinant MAGL is used to cleave an MAGL substrate, resulting in a fluorescence increase that is abrogated by the presence of an inhibitor for this enzyme. Specific inhibitors for this enzyme are of great clinical interest due to the central role for MAGL in lipid metabolism and energy storage.



II. Applications:

- Screening for inhibitors of human MAGL enzyme

III. Kit Contents:

Components	K474-100	Cap Code	Part Number
MAGL Assay Buffer	25 ml	WM	K474-100-1
MAGL Substrate (200X)	50 µl	Blue	K474-100-2
MAGL Enzyme (Human Recombinant)	1 vial	Green	K474-100-3
MAGL Control Inhibitor (JJKK-048)	100 µl	Orange	K474-100-4

IV. User Supplied Reagents and Equipment:

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

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **MAGL Assay Buffer:** Warm to room temperature before use. Store at -20ºC. 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.
- **MAGL Enzyme:** 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 Control Inhibitor (JJKK-048):** 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.

VI. Monoacylglycerol Lipase (MAGL) Inhibitor Screening Assay Protocol:

1. MAGL Enzyme Solution 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 MAGL Enzyme Solution to prepare). For each well, prepare a total of 90 µl MAGL Enzyme Solution, consisting of:

88 µl MAGL Assay Buffer
2 µl MAGL Enzyme 50X Stock Solution

Add 90 µl of the MAGL Enzyme Solution to each reaction well. Also prepare a background control (no enzyme) well by adding 95 µl MAGL Assay Buffer to an empty well.

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

a. Dissolve test compounds for screening into appropriate solvents to generate stock solutions. 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). 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).

Note: In our experience, DMSO has no appreciable effect on the activity of MAGL, 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).

b. 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. 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. A positive inhibition control well may also be prepared using the MAGL Control Inhibitor (JJKK-048). 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.

c. Preincubate the plate for 30 min at 37°C (protected from light), to allow test compounds to interact with MAGL.

3. **Substrate Addition:** 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**. Add 5 µl of the MAGL Substrate working solution (20X) to each reaction well, including background control (no enzyme) well(s).

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

4. **Measurement:** measure the fluorescence at Ex/Em = 360/460 nm in kinetic mode for 30-60 min at 37°C. While the assay can be performed in either endpoint or kinetic mode, we strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction.

5. **Calculation:** 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). Determine the change in fluorescence over the time interval for all reaction wells: $\Delta F/\Delta T = (RFU_2 - RFU_1) / (T_2 - T_1)$. 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. Relative activity can be calculated as follows:

$$\%\textbf{Relative Activity} = \frac{\textbf{Rate of Test Compound}}{\textbf{Rate of No Inhibitor Control}} \times \textbf{100}$$

Calculate %Relative Inhibition as follows:

$$\%\textbf{Relative Inhibition} = \frac{\textbf{Rate of No Inhibitor Control} - \textbf{Rate of Test Compound}}{\textbf{Rate of No Inhibitor Control}} \times \textbf{100}$$

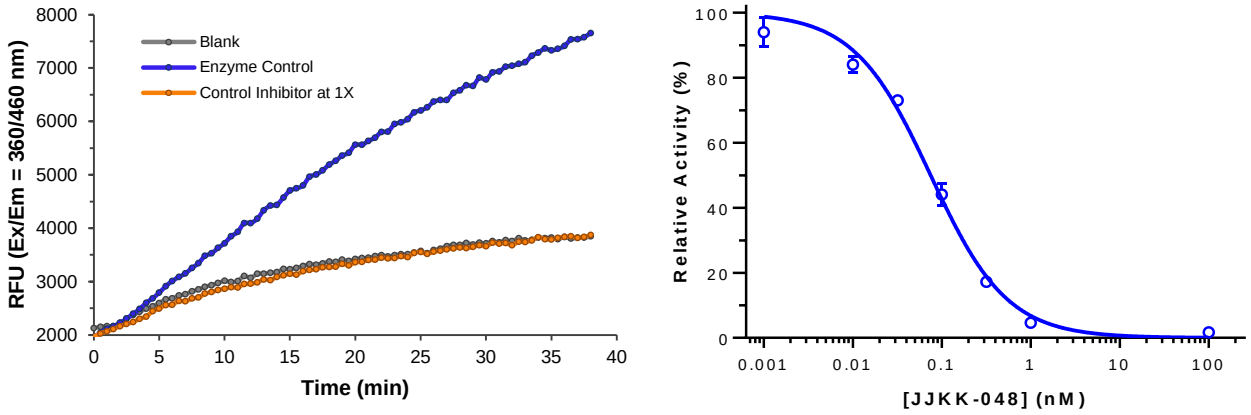


Figure: (a) Reaction kinetics of MAGL Enzyme in the presence and absence of JJKK-048 Control Inhibitor (10 µM). **(b)** IC_{50} determination for MAGL Control Inhibitor JJKK-048 (IC_{50} = 75 pM).

VII. RELATED PRODUCTS:

Arachidonic Acid (1505)
Total Bile Acids (TBA) Assay Kit (Colorimetric) (K209)
Triglyceride Quantification Assay Kit (K622)
Free Fatty Acid Quantification Colorimetric/Fluorometric Kit (K612)
EZScreen™ Triglyceride Assay Kit-384 Well Format (K952)
Cholesterol/Cholesteryl Ester Quantitation Kit (K603)
Cholesterol/Cholesteryl Ester Quantitation Assay Kit II (K623)
HDL and LDL/VLDL Quantitation Kit (K613)
PicoProbe™ Triglyceride Fluorometric Assay Kit (K614)
Cyclooxygenase (COX) Activity Assay Kit (Fluorometric) (K549)
JZL195 (B1064)

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