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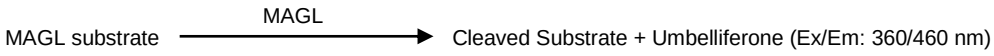
Monoacylglycerol Lipase (MAGL) Activity Assay Kit (Fluorometric)

(Catalog # K561-100; 100 assays; Store at -20°C)

I. Introduction:

Monoacylglycerol Lipase (MAGL, E.C. 3.1.1.23) is an important enzyme for regulation of endocannabinoid signaling in human physiology. MAGL is a serine hydrolase that generates free fatty acid and glycerol from monoacylglycerols such as 2-arachidonoyl glycerol (2-AG), one of the principal endocannabinoids active in the nervous system. One free fatty acid that is released by MAGL activity, arachidonic acid, is a precursor for eicosanoids, a family of pro-inflammatory signaling molecules. The leftover glycerol moiety can also serve various purposes as a metabolic building block and energy source. Inhibition or inactivation of MAGL leads to 2-AG buildup, and this has myriad biological consequences. Antinociceptive effects, anti-inflammatory and neuroprotective effects in Parkinson's and Alzheimer's diseases as well as reduced addiction withdrawal symptoms are some of the outcomes 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 activity assay kit provides a quick, sensitive and easy way for measuring MAGL activity in various samples. In this assay, a fluorescent substrate is cleaved to generate arachidonic acid and fluorescent metabolite and the increased fluorescence is measured at Ex/Em 360/460 nm. To identify the signal generated specifically by MAGL, a specific inhibitor is included that allows the user to differentiate MAGL activity from other sources of fluorescence. The assay is simple to perform, high-throughput adaptable and can detect as low as 0.1 mU of MAGL activity.



II. Applications:

- Measurement of monoacylglycerol lipase activity in various tissues/cells.

III. Sample Type:

- Animal tissues: brain, intestine etc.
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	K561-100	Cap Code	Part Number
MAGL Assay Buffer	25 ml	WM	K561-100-1
MAGL Substrate	50 µl	Blue	K561-100-2
MAGL Positive Control	1 vial	Green	K561-100-3
MAGL Selective Inhibitor (200X)	100 µl	Orange	K561-100-4
Umbelliferone Standard (10 mM)	40 µl	Yellow	K561-100-5

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom.
- Multi-well spectrophotometer (ELISA reader)

VI. 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. Keep the required amount at room temperature while in use. Aliquot the rest and store at -20°C. Avoid repeated freeze thaw cycles and protect from light. Use within two months.
- **Umbelliferone Standard:** Warm to room temperature and mix well before use. Aliquot if required and store at –20°C, protected from light. Use within two months.
- **MAGL Positive Control:** Reconstitute with 50 µl MAGL Assay Buffer. Aliquot and store at -20°C. Avoid repeated freeze thaw cycles and use within two months.
- **MAGL Selective Inhibitor :** Provided as a 200X stock solution in DMSO. Warm to room temperature before use. Aliquot and store at -20°C. Use within two months.

VII. Monoacylglycerol Lipase Assay Protocol:

Sample and Positive Control Preparation: Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold MAGL Assay Buffer and keep on ice for 10 min. Centrifuge at 10,000 x *g* at 4 ºC for 15 minutes and transfer the supernatant to a fresh tube. Since any given sample may produce background signal, you will need to designate two wells for each concentration of each sample. One will give the total signal, and the second will indicate background signal. To the **total signal wells,** add 5-40 µl sample per well & adjust the volume of the sample well to 90 µl with MAGL Assay Buffer**.** To the **background wells only**, add same amount of sample per well as used in the test wells to duplicate wells and adjust the volume to 85 µl with MAGL Assay Buffer. Dilute the MAGL Selective Inhibitor stock at a 1:10 ratio by diluting 5 µl of the 200X solution into 45 µl MAGL Assay Buffer, yielding a 20X working solution and add 5 µl of the 20X working solution to the **background wells (**sample + inhibitor). For the **MAGL Positive Control**: Add 5 µl of the **Positive Control** per well into the desired well(s) and adjust the volume to 90 µl with MAGL Assay Buffer. For the **Inhibitor Control**: add 80 µl of Assay Buffer to well, followed by 5 µl of the **Positive Control**. Add 2-5 µl of the **MAGL Control Inhibitor 20X working solution** to the inhibitor control well. Pre-incubate the plate for 20-30 minutes at 37°C (protected from light). This allows the inhibitor to act on the MAGL enzyme in the samples.

Notes:

a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

2. **Fluorescent Standard Curve:** Dilute the 10 mM Fluorescent standard solution 100 times by adding 10 µl of the standard solution to 990 µl MAGL Assay Buffer to obtain a 0.1 mM standard solution. Add 0, 4, 8, 12, 16 and 20 µl of 0.1 mM Fluorescent Standard into a series of wells in a 96 well black plate to generate 0, 0.4, 0.8, 1.2, 1.6 and 2 nmol/well of Fluorescent Standard. Adjust the volume to 100 µl/well with MAGL Assay Buffer.

3. **Substrate Mix:** During the pre-incubation period, prepare enough 10X working solution of MAGL Substrate by diluting the 200X stock in a 1:20 ratio **with Assay Buffer**. For instance, if running 5 experiments, requiring a total of 10 wells, dilute 5 µl **MAGL** Substrate Stock in 95 µl Assay Buffer. Add 10 µl of the MAGL Substrate working solution (10X) to each reaction well (total and background signal wells). Use the 10X substrate working solution within 2 hours.

Note:

a. *Do not add substrate to wells containing the standards*.

b. In our experience, DMSO has no appreciable effect on the activity of MAGL, even at concentrations as high as 10% (v/v).

4. **Measurement:** Measure fluorescence (Ex/Em= 360/460 nm) in kinetic mode for 60 min. at 37°C. The standard curve can be measured in end-point mode, and should not change substantially over the period of the kinetic assay.

Note: Measurement time for the linear phase of the reaction depends on the MAGL activity in samples. We recommend measuring the fluorescence in kinetic mode and choosing two time points (t_1 and t_2) in the linear range to calculate the MAGL activity of the samples.

5. **Calculation:** Subtract the 0 nmol Standard reading from all Standard Curve readings. Plot the Fluorescence Standard Curve. For all sample wells, subtract the reading of the sample + inhibitor wells (background signal) from their corresponding total activity wells to obtain the MAGL-specific signal of the samples (***F_S***). MAGL enzymatic activity is obtained by applying the ***F_S*** values to the Fluorescent standard curve to get *B* nmole of the fluorophore cleaved by MAGL enzyme during the reaction time ($\Delta t = t_2 - t_1$).

Sample Monoacylglycerol Lipase Activity = B/(Δt X V) x D = nmol/min/ml = mU/ml

Where: **B** = Umbelliferone amount from Standard Curve (nmol)
Δt = reaction time (min)
V = sample volume added into the reaction well (ml)
D = Dilution Factor

Unit Definition: One unit of **Monoacylglycerol Lipase** is the amount of enzyme that generates 1.0 µmole of umbelliferone per min at pH 7.5 at 37°C.

a) b) c)

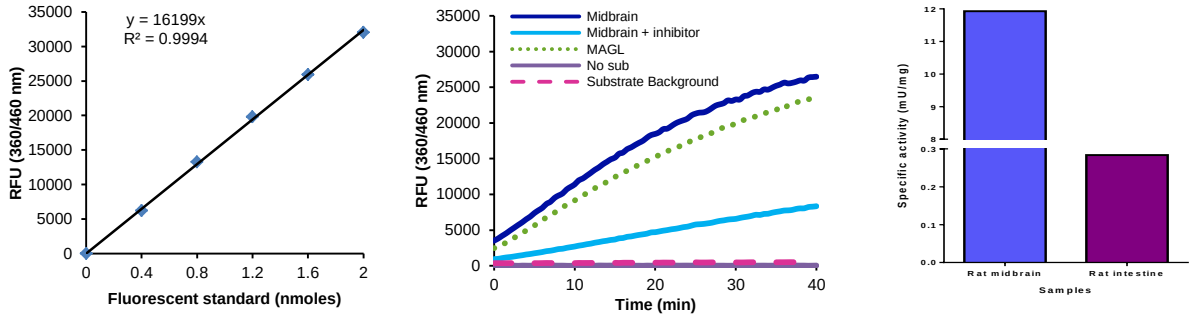


Figure: (a) Fluorescent standard curve; (b) Reaction kinetics of Monoacylglycerol Lipase (MAGL) positive control and MAGL activity in rat midbrain lysate (6 µg protein) using appropriate background controls; (c) Monoacylglycerol Lipase specific activity was calculated in rat midbrain and rat intestinal lysates. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

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

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