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ab273326 Monoacylglycerol Lipase (MAGL) Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273326
(use https://www.abcam.cn/ab273326 for china, or
https://www.abcam.co.jp/ab273326 for Japan)

For the determination of MAGL activity in cell and tissue lysates.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Monoacylglycerol Lipase (MAGL) Activity Assay Kit (Fluorometric) (ab273326) 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.

2. Protocol Summary

Prepare samples and add to wells.

Prepare controls and add to wells.

Incubate plate for 20-30 mins at 37°C protected from light to allow inhibitor to act.

Prepare standards and add to wells.

Add Substrate Mix to appropriate wells.

Measure fluorescence in kinetic mode for 60 mins at 37°C.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Mutant IDH Lysis Buffer	25 mL	-20°C
MAGL Substrate	50 µL	-20°C
MAGL Positive Control	1 vial	-20°C
MAGL Inhibitor	100 µL	-20°C
Umbelliferone Standard	40 µL	-20°C

7. Materials Required, Not Supplied

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

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

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Mutant IDH Lysis Buffer:**
Warm to room temperature before use. Store at -20°C. Use within two months.

9.2 **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.

9.3 **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.

9.4 **MAGL Positive Control:**
Reconstitute with 50 µl Mutant IDH Lysis Buffer. Aliquot and store at -20°C. Avoid repeated freeze thaw cycles and use within two months.

9.5 **MAGL 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.

10. Sample Preparation

Sample Preparation:

10.1 Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold Mutant IDH Lysis Buffer/MAGL Assay Buffer and keep on ice for 10 mins.

10.2 Centrifuge at 10,000 x g at 4 °C for 15 mins 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.

10.3 One will give the total signal, and the second will indicate background signal.

10.4 To the total signal wells, add 5-40 µl sample per well and adjust the volume of the sample well to 90 µl with Mutant IDH Lysis Buffer.

10.5 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 Mutant IDH Lysis Buffer.

10.6 Dilute the MAGL Inhibitor stock at a 1:10 ratio by diluting 5 µl of the 200X solution into 45 µl Mutant IDH Lysis Buffer, yielding a 20X working solution and add 5 µl of the 20X working solution to the background wells (sample + MAGL Inhibitor.

Control Preparations:

10.7 Add 5 µl of the Positive Control per well into the desired well(s) and adjust the volume to 90 µl with Mutant IDH Lysis Buffer.

10.8 For the Inhibitor Control: add 80 µl of Mutant IDH Lysis Buffer to well, followed by 5 µl of the Positive Control.

10.9 Add 2-5 µl of the MAGL Control Inhibitor 20X working solution to the inhibitor control well.

10.10 Pre-incubate the plate for 20-30 mins at 37°C (protected from light). This allows the inhibitor to act on the MAGL enzyme in the samples.

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

11. Standard Curve

11.1 Dilute the 10 mM Fluorescent standard solution 100 times by adding 10 µl of the standard solution to 990 µl Mutant IDH Lysis Buffer to obtain a 0.1 mM standard solution.

11.2 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.

11.3 Adjust the volume to 100 µl/well with Mutant IDH Lysis Buffer/MAGL Assay Buffer.

Standard #	0.1 mM Fluorescent - Standard (µL)	Mutant IDH Lysis Buffer (µL)	Fluorescent Standard (nmol/well)
1	20	80	2
2	16	84	1.6
3	12	88	1.2
4	8	92	0.8
5	4	96	0.4
6	0	100	0

12. Assay Procedure

Substrate mix:

12.1 During the pre-incubation period, prepare enough 10X working solution of MAGL Substrate by diluting the 200X stock in a 1:20 ratio with Mutant IDH Lysis Buffer. For instance, if running five experiments, requiring a total of 10 wells, dilute 5 µl MAGL Substrate Stock in 95 µl Mutant IDH Lysis Buffer.

12.2 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**: Do not add substrate to wells containing the standards.

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

12.3 Measure fluorescence (Ex/Em = 360/460 nm) in kinetic mode for 60 mins at room temperature.

12.4 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 (t1 and t2) in the linear range to calculate the MAGL activity of the samples.

13. Calculations

13.1 Subtract the 0 nmol Standard reading from all Standard Curve readings. Plot the Fluorescence Standard Curve.

13.2 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 (FS).

13.3 MAGL enzymatic activity is obtained by applying the FS values to the Fluorescent standard curve to get B nmole of the fluorophore cleaved by MAGL enzyme during the reaction time ($\Delta t = t2 - t1$).

$$Sample\ MAGL\ activity\ = \frac{B}{(\Delta t \times V)} \times D = (nmol/min/ml) = mU/mL$$

B = Umbelliferone amount from Standard Curve (nmol)
Δt = Reaction time (mins)
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.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

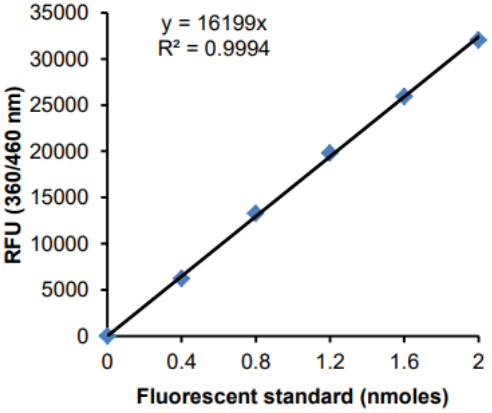

Figure 1. Fluorescent standard curve.

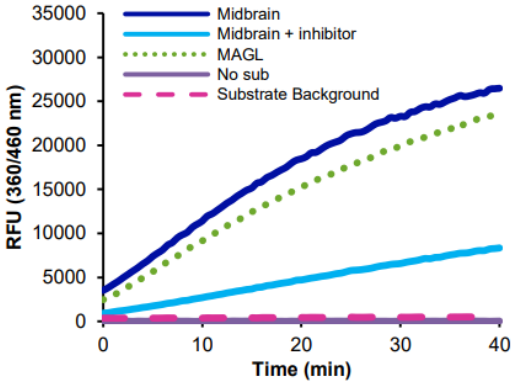

Figure 2. Reaction kinetics of Monoacylglycerol Lipase (MAGL) positive control and MAGL activity in rat midbrain lysate (6 µg protein) using appropriate background controls.

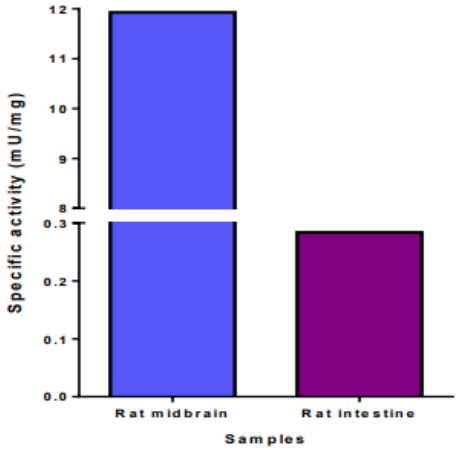

Figure 3. Monoacylglycerol Lipase specific activity was calculated in rat midbrain and rat intestinal lysates.

15. FAQ / Troubleshooting

General troubleshooting points are found at https://www.abcam.com/en-us/products/biochemical-assays.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)