

**Monoacylglycerol Lipase Inhibitor  
Screening Assay Kit**

Item No. 705192

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## GENERAL INFORMATION

### Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity	Storage
705193	MAGL Substrate	1 vial	-20°C
705194	MAGL (human recombinant)	1 vial	-80°C
705195	MAGL Assay Buffer (10X)	1 vial	-20°C
400014	96-Well Plate (Colorimetric Assay)	1 plate	Room temperature
400012	96-Well Cover Sheet	1 cover	Room temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



**WARNING:** This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

## Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

## If You Have Problems

### Technical Service Contact Information

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**Fax:** 734-971-3641

**Email:** techserv@caymanchem.com

**Hours:** M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as specified in the **Materials Supplied** section and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 405-415 nm
2. Adjustable pipettes and a repeat pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

## INTRODUCTION

### Background

The endocannabinoid system is a ubiquitous lipid signaling system that is involved in various regulatory functions throughout the body. The main endocannabinoids are arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG). They bind to G protein-coupled receptors, of which the cannabinoid (CB<sub>1</sub>) receptor is densely distributed in areas of the brain related to motor control, cognition, emotional responses, and homeostasis.<sup>1-4</sup> Acting *via* the CB<sub>2</sub> receptor in the peripheral tissues, the endocannabinoid system is one of the crucial modulators of the autonomic nervous system, the immune system, and microcirculation. Endocannabinoids are released upon demand from lipid precursors in a receptor-dependent manner. They are transported into cells by an apparently specific uptake system and degraded primarily by two enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) resulting in the termination of their biological actions.<sup>5</sup> FAAH, a serine hydrolase, can degrade many fatty acid amides, including AEA. Although FAAH can hydrolyze 2-AG, the main enzyme responsible for the inactivation of this monoglyceride is another serine hydrolase, MAGL. Finding inhibitors to these endocannabinoid hydrolases could offer another approach in the treatment of pain, obesity, and various neurological diseases, where higher endocannabinoid activity would be beneficial. An advantage of such enzyme inhibition over direct CB agonists could result in higher selectivity, as it would increase activity of the endocannabinoid system only at sites where on-going production of endocannabinoids is taking place.<sup>6</sup>

### About This Assay

Cayman's Monoacylglycerol Lipase Inhibitor Screening Assay provides a convenient method for screening human MAGL inhibitors. MAGL hydrolyzes 4-nitrophenylacetate resulting in a yellow product, 4-nitrophenol, with an absorbance of 405-412 nm.<sup>7</sup>

## Reagent Preparation

### 1. MAGL Substrate - (Item No. 705193)

The vial contains 1.2 ml of a 4.25 mM ethanolic solution of 4-nitrophenylacetate. The Substrate is ready to use in the assay. *NOTE: The final concentration of Substrate in the assay as described below is 236  $\mu$ M. This concentration may be reduced with ethanol at the user's discretion, particularly when complete inhibition curves are required for  $IC_{50}$  or  $K_i$  determination. For competitive inhibitors, the  $IC_{50}$  of an inhibitor is dependent upon the substrate concentration and should be reported when publishing the experimental results. The  $K_m$  for the Substrate is 500  $\mu$ M.*

### 2. MAGL (human recombinant) - (Item No. 705194)

This vial contains 100  $\mu$ l of a solution of human recombinant MAGL (monoacylglycerol lipase). The thawed enzyme should be stored on ice. Dilute 30  $\mu$ l of enzyme with 570  $\mu$ l of diluted MAGL Assay Buffer. This is enough enzyme to assay 50 wells. Dilute additional enzyme if using the entire plate. The diluted enzyme is stable for four hours on ice.

### 3. MAGL Assay Buffer (10X) - (Item No. 705195)

Dilute 3 ml of Assay Buffer concentrate with 27 ml of HPLC-grade water. This final Assay Buffer (10 mM Tris-HCl, pH 7.2, containing 1 mM EDTA) should be used in the assay and for the dilution of MAGL. The diluted Assay Buffer is stable for at least six months if stored at -20°C.

## Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% Initial Activity wells and three wells designated as Background wells. We suggest that each inhibitor sample be assayed in triplicate and that you record the contents of each well on the template sheet provided on page 15. A typical layout of samples and inhibitors to be measured in triplicate is shown in Figure 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	BW	7	7	7	15	15	15	23	23	23
B	A	A	A	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

BW - Background Wells

A - 100% Initial Activity Wells

1-30 - Inhibitor Wells

Figure 1. Inhibitor screening plate format

### Pipetting Hints

- It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

### General Information

- The final volume of the assay is 180  $\mu\text{l}$  in all the wells.
- It is not necessary to use all the wells on the plate at one time.
- If the appropriate inhibitor dilution is not known, it may be necessary to assay at several dilutions.
- It is recommended that the samples be assayed in triplicate, but this is left to the user's discretion.
- Thirty inhibitor samples can be assayed in triplicate or forty-five in duplicate.
- Monitor the absorbance at 405-415 nm using a plate reader.

### Performing the Assay

1. **100% Initial Activity Wells** - add 150  $\mu\text{l}$  of diluted Assay Buffer, 10  $\mu\text{l}$  of MAGL, and 10  $\mu\text{l}$  of solvent (same solvent used to dissolve the inhibitor) to three wells.
2. **Background Wells** - add 160  $\mu\text{l}$  of diluted Assay Buffer and 10  $\mu\text{l}$  of solvent (same solvent used to dissolve the inhibitor) to three wells.
3. **Inhibitor Wells** - add 150  $\mu\text{l}$  of diluted Assay Buffer, 10  $\mu\text{l}$  of MAGL, and 10  $\mu\text{l}$  of inhibitor\* to three wells.

	Assay Buffer	MAGL	Solvent	Inhibitor
100% Initial Activity	150 $\mu\text{l}$	10 $\mu\text{l}$	10 $\mu\text{l}$	-
Background	160 $\mu\text{l}$	-	10 $\mu\text{l}$	-
Inhibitor	150 $\mu\text{l}$	10 $\mu\text{l}$	-	10 $\mu\text{l}$

4. Initiate the reactions by adding 10  $\mu\text{l}$  of MAGL Substrate to all the wells being used. Carefully shake the microtiter plate for 10 seconds to mix and cover with the plate cover. Incubate for ten minutes at room temperature.
5. Remove the plate cover and read the absorbance at 405-415 nm.

\*Inhibitors can be dissolved in methanol, dimethylsulfoxide, or ethanol and should be added to the assay in a final volume of 10  $\mu\text{l}$ . In the event that an appropriate concentration of inhibitor is completely unknown, we recommend that several dilutions of the inhibitor be made.

## ANALYSIS

### Calculations

1. Determine the average absorbance of the background, initial activity, and inhibitor wells.
2. Subtract the average absorbance of the background wells from the average absorbance of the 100% initial activity and the inhibitor wells.
3. Determine the percent inhibition or percent Initial Activity for each sample.

$$\% \text{ Inhibition} = \left[ \frac{\text{IA} - \text{Inhibitor}}{\text{IA}} \right] \times 100$$

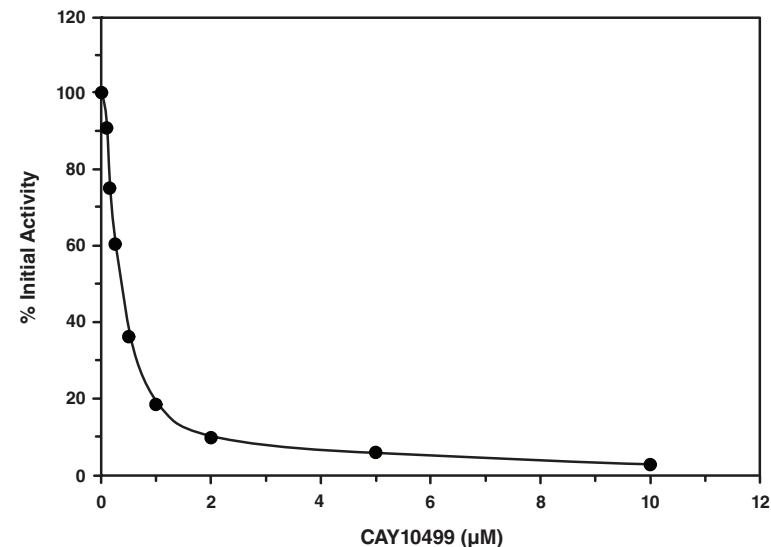
$$\% \text{ Initial Activity} = \frac{\text{Inhibitor}}{\text{IA}} \times 100$$

4. Graph the percent inhibition or percent initial activity as a function of the inhibitor concentration to determine the  $IC_{50}$  value (concentration at which there was 50% inhibition). The inhibition of human recombinant MAGL by CAY10499 is shown in Figure 2 (see page 11) as an example.<sup>7</sup>

Name	Item No.	$IC_{50}$ value
IDFP	10215	7 nM
JZL184	13158	1.5 $\mu$ M
MAFP	70660	17 nM
CAY10499	10007875	350 nM

**Table 1.  $IC_{50}$  values of MAGL inhibitors as determined using this assay**

*NOTE: The MAGL inhibitors, URB602 and URB754, do not work in the assay because they do not stay in solution.*



**Figure 2. Inhibition of human recombinant MAGL by CAY10499 ( $IC_{50} = 350$  nM)**

### Performance Characteristics

#### Precision:

When a series of 16 MAGL measurements were assayed on the same day, the intra-assay coefficient of variation was 1.4%. When a series of 16 MAGL measurements were assayed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 2.1%.

## Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No absorbance above background is seen in the Inhibitor wells.	A. Enzyme or substrate was not added to the well(s) B. Inhibitor concentration is too high and inhibited all of the enzyme activity	A. Make sure to add all components to the wells B. Reduce the concentration of the inhibitor
No inhibition seen with inhibitor.	A. The inhibitor concentration is not high enough B. The compound is not an inhibitor of the enzyme	Increase the inhibitor concentration and re-assay

## References

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3. Kondo, S., Kondo, H., Nakane, S., *et al.* 2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: Identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through Ca<sup>2+</sup>-dependent and -independent mechanisms. *FEBS Lett.* **429**, 152-156 (1998).
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6. Lambert, D.M. and Fowler, C.J. The endocannabinoid system: Drug targets, lead compounds, and potential therapeutic applications. *J. Med. Chem.* **48(16)**, 5059-5087 (2005).
7. Muccioli, G.G., Labar, G., and Lambert, D.M. CAY10499, a novel monoglyceride lipase inhibitor evidenced by an expeditious MGL assay. *ChemBioChem* **9**, 2704-2710 (2008).

## Related Product

FAAH Inhibitor Screening Assay Kit - Item No. 10005196

## Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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## NOTES

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