



## Monoacylglycerol Lipase Inhibitor Screening Assay Kit

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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
700307	JZL 195 Inhibitor Assay Reagent	1 vial	-20°C
400014	96-Well Plate (Colorimetric Assay)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this assay.**

## 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

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 directed 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; multichannel or repeating pipettor are recommended
3. A source of ultrapure water is recommended. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. DMSO to resuspend the JZL 195 positive control inhibitor

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

Dilute 3 ml of MAGL Assay Buffer (10X) concentrate with 27 ml of ultrapure water. This final 1X 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 enzyme, substrate, and JZL 195 inhibitor. The 1X Assay Buffer is stable for at least six months if stored at -20°C.

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

The vial contains 0.5 ml of 17 mM ethanolic solution of 4-nitrophenylacetate. Mix 150 µl of this solution with 450 µl of 1X Assay Buffer prior to testing. This is enough substrate to assay 50 wells. Dilute additional substrate if using the entire plate. *NOTE: The final concentration of Substrate in the assay as described below is 236 µM. The K<sub>m</sub> for the Substrate is 300 µM.*

### 3. MAGL (human, recombinant) - (Item No. 705194)

This vial contains 100 µl of a solution of human recombinant MAGL (monoacyl-glycerol lipase). The thawed enzyme should be stored on ice. Dilute 30 µl of enzyme with 570 µl of 1X 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.

### 4. JZL 195 Inhibitor Assay Reagent - (Item No. 700307)

This vial contains 20 nmol of inhibitor. Resuspend in 125 µl of DMSO, then add 125 µl of 1X Assay Buffer and vortex to make a 80 µM stock. The addition of 10 µl to the assay yields a final concentration of 4.4 µM inhibitor in the well. JZL 195 Inhibitor Assay Reagent can be used as a positive control in the assay.

## ASSAY PROTOCOL

### Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. 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. It is suggested that each inhibitor, including the positive control JZL 195 inhibitor assay reagent, be assayed in triplicate. It is suggested that the contents of each well be recorded on the template sheet provided on page 21. 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. Sample 96-well format

### Pipetting Hints

- It is recommended that a multichannel 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  $\mu$ l in all the wells.
- It is not necessary to use all the wells on the plate at one time.
- 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.
- Use the 1X Assay Buffer in the assay.
- The assay is performed at room temperature.

## Performing the Assay

- 100% Initial Activity Wells** - add 150  $\mu\text{l}$  of 1X Assay Buffer, 10  $\mu\text{l}$  of MAGL enzyme, and 10  $\mu\text{l}$  of solvent (same solvent used to dissolve the inhibitor) to three wells. If multiple inhibitors in different solvents are to be assayed at the same time, separate sets of 100% initial activity wells should be run for each solvent.
- Background Wells** - add 160  $\mu\text{l}$  of 1X Assay Buffer and 10  $\mu\text{l}$  of solvent (same solvent used to dissolve the inhibitor) to three wells.
- Inhibitor/Positive Control Wells** - add 150  $\mu\text{l}$  of 1X Assay Buffer, 10  $\mu\text{l}$  of MAGL enzyme, and 10  $\mu\text{l}$  of inhibitor\* or positive control JZL 195 inhibitor to the inhibitor wells.

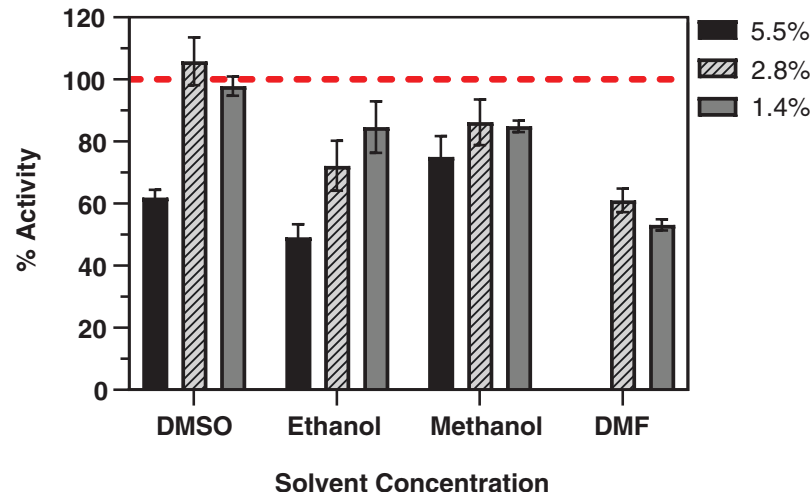
	1X Assay Buffer	MAGL	Solvent	Inhibitor
100% Initial Activity	150 $\mu\text{l}$	10 $\mu\text{l}$	10 $\mu\text{l}$	-
Background	160 $\mu\text{l}$	-	10 $\mu\text{l}$	-
Inhibitor	150 $\mu\text{l}$	10 $\mu\text{l}$	-	10 $\mu\text{l}$

- Mix the contents of the wells by pipetting gently up and down and incubate for 15 minutes at room temperature. The incubation time for different inhibitors may vary and will need to be optimized by the user.
- Initiate the reactions by adding 10  $\mu\text{l}$  of MAGL Substrate to all the wells. Carefully shake the 96-well plate for 10 seconds to mix and cover with the plate cover. Incubate for ten minutes at room temperature.
- Remove the plate cover and read the absorbance at 405-415 nm.

\*Inhibitors can be dissolved in a solvent of your choice (see Fig. 2) and should be added to the assay in a final volume of 10  $\mu\text{l}$ .

## Effects of Solvents:

Compounds may be prepared in organic solvents such as DMSO, DMF, or short-chain alcohols (e.g. MeOH, EtOH), as long as the final concentration of organic solvents in the assay is  $\leq 2.8\%$  DMSO and 1.4% Ethanol and Methanol. DMF significantly decreases enzyme activity and is not recommended for use in the assay.



**Figure 2. The effect of solvents on MAGL activity.** The red line corresponds to the activity of MAGL enzyme in the Assay buffer (1X) without any solvents.

## Calculations

1. Determine the average absorbance of each sample.
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 activity for each inhibitor using one of the following equations:

$$\% \text{inhibition} = \left[ \frac{(\text{corrected 100\% initial activity} - \text{corrected inhibitor activity})}{\text{corrected 100\% initial activity}} \right] \times 100$$

$$\% \text{ activity} = \left[ \frac{(\text{corrected inhibitor activity})}{\text{corrected 100\% initial activity}} \right] \times 100$$

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 is 50% inhibition). The inhibition of human recombinant MAGL by JZL 195 is shown in Figure 3 (see page 14) as an example.<sup>7</sup>

Name	Item No.	$IC_{50}$ value
JZL 184	13158	20 nM
CAY10499	10007875	50 nM
MAGL 23	27348	87 nM

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

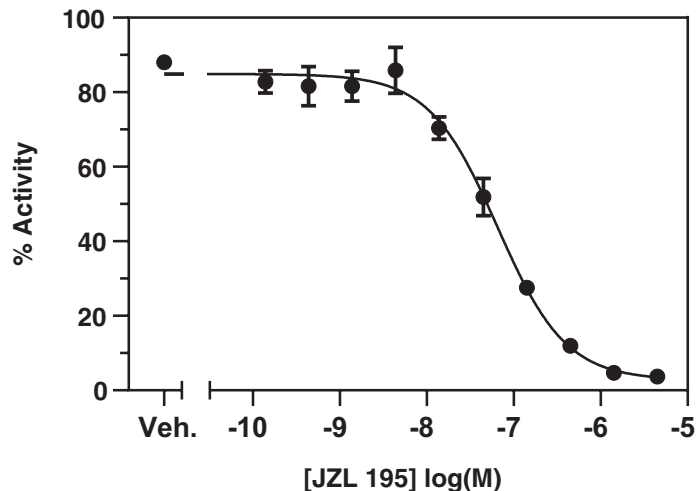


Figure 3. Inhibition of human recombinant MAGL by JZL 195 ( $IC_{50} = 65$  nM)

## Performance Characteristics

### Z' Factor:

Z' factor is a term used to describe the robustness of an assay, which is calculated using the equation below.<sup>8</sup>

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{|\mu_{c+} - \mu_{c-}|}$$

Where  $\sigma$ : Standard deviation

$\mu$ : Mean

c+: Positive control

c-: Negative control

The theoretical upper limit for the Z' factor is 1.0. A robust assay has a Z' factor >0.5. The Z' factor for Cayman's Monoacylglycerol Lipase Inhibitor Screening Assay Kit was determined to be 0.80.



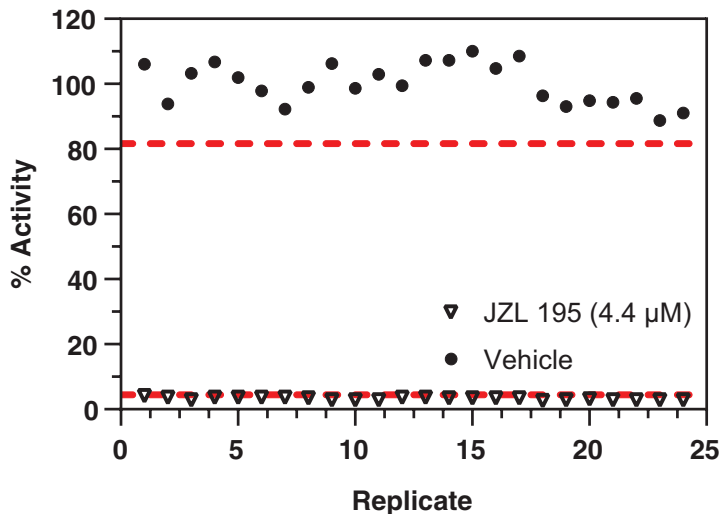


Figure 4. Typical Z' data for the Monoacylglycerol Lipase Inhibitor Screening Assay Kit. Data are shown from wells of both positive and negative controls prepared as described in the kit booklet. The calculated Z' factor from this experiment was 0.80. The red lines correspond to three standard deviations from the mean for each control value.

#### Precision:

Intra-assay precision was determined by analyzing 24 measurements of the background, vehicle, and 4.4 μM JZL 195 inhibitor assay reagent on the same day. The intra-assay coefficients of variation were 2.0, 5.4, and 2.4%, respectively. The intra-assay coefficient of variation for the IC<sub>50</sub> value of 5 inhibition curves performed on the same day was 13.8%.

Inter-assay precision was determined by analyzing inhibition with JZL 195 in 5 separate assays on different days. The inter-assay coefficient of variance for the IC<sub>50</sub> value was 10.1%.

## 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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### Warranty and Limitation of Remedy

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