Caspase-1 Inhibitor Screening Assay Kit

Item No. 701840

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Customer Service 800.364.9897
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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
701841	Caspase Assay Buffer (5X)	1 vial/5 ml	-20°C
700416	DTT (1M) Assay Reagent	1 vial/1 ml	-20°C
701842	Caspase-1 Substrate (Ac-YVAD-AFC)	1 vial/50 µl	-20°C
701843	Caspase-1 Enzyme (human, recombinant)	1 vial/20 µl	-80°C
701844	Caspase-1 Inhibitor (Ac-YVAD-CHO)	1 vial/40 µl	-20°C
400093	384-Well Solid Plate (low volume; black)	1 plate	RT
400023	Foil Plate Cover	1 ea	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.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3640

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 (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure fluorescence with excitation and emission wavelengths of 400 and 505 nm, respectively
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass-distilled water or deionized water is acceptable
   NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).

INTRODUCTION

Background

Caspase-1, also known as interleukin-1β converting enzyme (ICE), is a cysteinyl aspartic protease that is required for pyroptosis, a lytic, inflammatory form of cell death characterized by the secretion of IL-1β and IL-18, *via* the canonical inflammasome pathway.[1] Caspase-1 is activated by cleavage of the N-terminal caspase activation and recruitment domain (CARD) of procaspase-1 by the inflammasome, a multimeric protein complex formed following the detection of various pathogen- or damage-associated molecular patterns (PAMPs or DAMPs) by pattern-recognition receptors such as NLRP1, NLRC4, NLRP3, NLRP6, AIM2, or IF116.[1,2] Upon activation, caspase-1 cleaves pro-IL-1β and pro-IL-18 into active IL-1β and IL-18, which are secreted from the cell. It also activates gasdermin D, a pore-forming effector protein necessary for pyroptosis, and stimulates the release of factors involved in tissue repair.[3,4] Mice lacking caspase-1, paradoxically, have increased inflammation and cytokine levels, as well as higher mortality in response to viral stimuli.[5] Caspase-1 has a complex effect on fat mass.[6,7] Knockout of caspase-1 reduces fat mass in mice fed a normal diet compared to wild-type animals.[6] However, in caspase-1 knockout mice fed a high-fat diet, fat mass is increased in a sex-dependent manner, with male mice gaining extra fat mass sooner than female mice.[7] In addition, caspase-1 knockout reduces hepatic steatosis and increases in plasma cholesterol and fatty acid levels in a mouse model of high-fat diet-induced non-alcoholic steatohepatitis (NASH).[8] In humans, mutations in caspase-1 reduce the expression or enzymatic activity of the enzyme, and variants that decrease caspase-1 activity are associated with increased activation of NF-κB and an inflammatory phenotype.[9,10] Pharmacological inhibition of caspase-1 by VX-740 reduces joint damage in mouse models of osteoarthritis and prevents colitis development in mice.[11,12] Development of improved caspase-1 inhibitors is essential for determining the effects of caspase-1 inhibition in cellular and animal models of inflammation and for their potential use in the treatment of inflammation-associated diseases.

About This Assay

Cayman's Caspase-1 Inhibitor Screening Assay Kit provides a robust and easy-to-use platform for identifying novel inhibitors of human caspase-1, the major downstream effector following inflammasome activation. The assay uses a caspase-1-specific fluorogenic substrate, Ac-YVAD-AFC. Caspase-1 cleaves this substrate generating free AFC, which can be easily quantified using a fluorescence plate reader at excitation and emission wavelengths of 400 and 505 nm, respectively. The potent and reversible caspase-1 inhibitor Ac-YVAD-CHO is included as a positive control.

PRE-ASSAY PREPARATION

Sample Preparation

All inhibitors, be they small molecules, natural products, or proteins, should be prepared in diluted Caspase Assay Buffer at a concentration 4X the desired final assay concentration (*e.g.*, for 1 µM final assay concentration, a 4 µM stock should be made). This solution may contain up to 8% DMSO, DMF, or short-chain alcohols (*e.g.*, MeOH, EtOH). The final concentration of organic solvents in the assay will then be ≤2% (see 'Effects of Solvents' on page 17).

Reagent Preparation

1. **Caspase Assay Buffer (1X)**

   Mix 2 ml of Caspase Assay Buffer (5X) (Item No. 701841) with 7.9 ml of water and 100 µl of the supplied DTT (1 M) Assay Reagent (Item No. 700416) to make 10 ml of Caspase Assay Buffer (1X). The Caspase Assay Buffer (1X) should be discarded if not used within the same day. Once thawed, the Caspase Assay Buffer (5X) may be stored at 4°C for at least one month.

2. **Caspase-1 Substrate (Ac-YVAD-AFC)**

   This vial contains Caspase-1 Substrate (Ac-YVAD-AFC) (Item No. 701842) in DMSO. Mix 20 µl of Caspase-1 Substrate (Ac-YVAD-AFC) with 3.98 ml Caspase Assay Buffer (1X). The diluted substrate will be stable at room temperature for 4 hours. If all of the Caspase-1 Substrate (Ac-YVAD-AFC) will not be used at one time, aliquot the undiluted substrate and store at -20°C where it will be stable for at least one month.

3. **Caspase-1 Enzyme (human, recombinant)**

   Caspase-1 Enzyme (human, recombinant) (Item No. 701843) should be thawed on ice and mixed prior to dilution. To dilute the enzyme, mix 16 µl of Caspase-1 Enzyme (human, recombinant) with 1.984 ml Caspase Assay Buffer (1X). It is recommended that the enzyme be diluted immediately prior to performing the assay. The diluted enzyme loses 30% of its activity when stored on ice for one hour. The undiluted enzyme can be stored at -80°C, limiting freeze-thaw cycles.

4. **Caspase-1 Inhibitor (Ac-YVAD-CHO)**

   This vial contains Caspase-1 Inhibitor (Ac-YVAD-CHO) (Item No. 701844) in DMSO, which can be used as a positive control. Mix 5 µl of Caspase-1 Inhibitor with 45 µl of Caspase Assay Buffer (1X) to make a 100 µM stock solution. Then mix 4 µl of the 100 µM Caspase-1 Inhibitor stock solution with 96 µl Caspase Assay Buffer (1X) to make a 4 µM working solution. If all of the Caspase-1 Inhibitor will not be used at one time, aliquot the undiluted inhibitor and store at -20°C.

ASSAY PROTOCOL

Plate Set Up

The 384-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 and three wells designated as background. It is suggested that each inhibitor (including the positive control Caspase-1 Inhibitor) be assayed in triplicate.

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- 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 20 µl in all the wells.
- Use the diluted assay buffer in the assay.
- All reagents should be prepared as described above and kept at room temperature before beginning the assay, except Caspase-1 Enzyme (human, recombinant).
- It is not necessary to use all the wells on the plate at one time.
- If the appropriate inhibitor concentration is not known, it may be necessary to assay at several concentrations.
- It is recommended to assay the samples in triplicate, but it is the user's discretion to do so.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength of 400 nm and an emission wavelength of 505 nm.

Performing the Assay

1. **Background Wells**: add 10 µl Caspase Assay Buffer (1X) to three wells.
2. **100% Initial Activity Wells**: add 5 µl of the diluted Caspase-1 Enzyme (human, recombinant) and 5 µl of solvent to three wells. Use the same solvent concentration used for the unknown inhibitor and the positive control, Caspase-1 Inhibitor (Ac-YVAD-CHO).
3. **Inhibitor/Positive Control Wells**: add 5 µl of Caspase-1 Enzyme (human, recombinant) and 5 µl of unknown inhibitor or the 4 µM positive control (Caspase-1 Inhibitor) working solution to three wells. If 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. *NOTE: To determine an IC_{50} value for an inhibitor, multiple concentrations of the inhibitor should be tested in the assay.*
4. Incubate for 10 minutes at room temperature.
5. Initiate the reactions by adding 10 µl of Caspase-1 Substrate (Ac-YVAD-AFC) to all the wells being used. Mixing the contents is not necessary.
6. Cover the plate with the Foil Plate Cover (Item No. 400023) and incubate for two hours at room temperature.
7. Remove the plate cover and read the plate with an excitation wavelength of 400 nm and an emission wavelength of 505 nm.

ANALYSIS

Calculations

1. Determine the average fluorescence (AF) of each sample.
2. Subtract the AF of the background wells from the AF of the 100% initial activity and inhibitor wells. These are the corrected values.
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 activity as a function of inhibitor concentration to determine the IC_{50} value (the concentration at which there is 50% inhibition) of the inhibitor. Inhibition of recombinant human caspase-1 by Caspase-1 Inhibitor (Ac-YVAD-CHO) is shown in Figure 1 (see page 15).

Performance Characteristics

Z´ Factor:

Z´ factor is a term used to describe the robustness of an assay, which is calculated using the equation below.[13]

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

Where σ: Standard deviation
μ: 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 Caspase-1 Inhibitor Screening Assay Kit was determined to be 0.79.

Sample Data:

The data shown here is an example of the data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to directly compare to your samples. Your results could differ substantially.

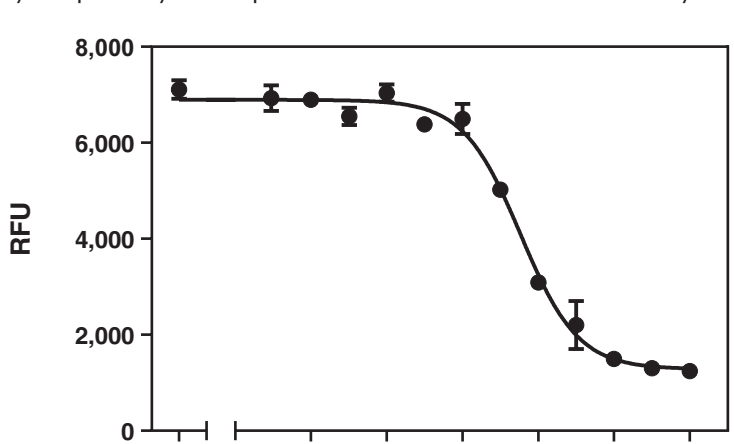

Figure 1. Inhibition of recombinant human caspase-1 by Caspase-1 Inhibitor (Ac-YVAD-CHO). Data are plotted as the mean of triplicate measurements ± the standard deviation. The vehicle control (Veh.) represents 100% initial activity.

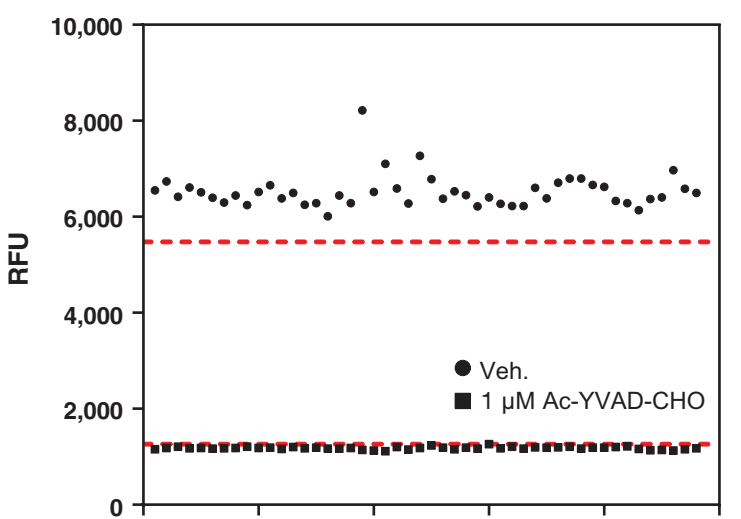

Figure 2. Typical Z′ data for the Caspase-1 Inhibitor Screening Assay Kit. Data are shown from 48 replicates each for vehicle control (Veh.) and 1 µM Caspase-1 Inhibitor (Ac-YVAD-CHO) prepared as described in the kit booklet. The calculated Z′ factor for this experiment was 0.79. The red lines correspond to three standard deviations from the mean for each control value.

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 ≤2%. A titration of organic solvents showed that the signal increases slightly with increasing solvent concentration so the proper vehicle control should be included in the assay.

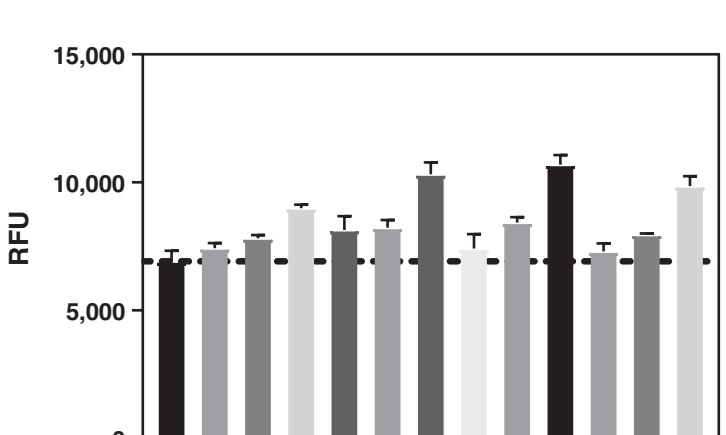

Figure 3. The effect of solvent on the readout of caspase-1 activity. The data are shown as the mean ± standard deviation for triplicate reactions containing the indicated concentration of solvents.

Precision:

Intra-assay precision was determined by analyzing 10-24 measurements of the background, vehicle, and 1 µM Caspase-1 Inhibitor (Ac-YVAD-CHO) on the same day. The intra-assay coefficients of variation were 3.7, 4.8, and 5.2%, respectively. The intra-assay coefficient of variation for the IC_{50} value of 12 inhibition curves performed on the same day was 12.4%.

Inter-assay precision was determined by analyzing inhibition with Caspase-1 Inhibitor (Ac-YVAD-CHO) in separate assays on three different days. The inter-assay coefficient of variance for the IC_{50} value was 9.6%.

RESOURCES

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 fluorescence detected above background 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 the components to the well(s) B. Reduce the inhibitor concentration and re-assay
The fluorometer exhibited 'MAX' values for the wells	The *gain* setting is too high	Reduce the *gain* and re-read
No inhibition seen with compound	A. The compound concentration is not high enough B. The compound is not an inhibitor of the enzyme	Increase the compound concentration and re-assay

References

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NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.