

**ab102496**

# **Caspase 8 Inhibitor Drug Detection Kit**

## **Instructions for Use**

For the rapid, sensitive and accurate detection of Caspase 8 inhibition by various compounds

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

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# 1. Overview

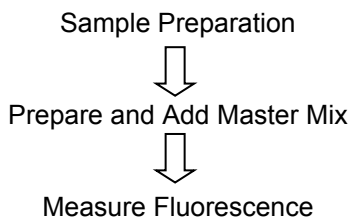
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Caspases have been shown to play a crucial role in apoptosis induced by various deleterious and physiologic stimuli. Inhibition of caspases can delay apoptosis, implicating a potential role in drug screening efforts.

Abcam's Caspase 8 Inhibitor Drug Detection Kit provides an effective means for screening caspase inhibitors using fluorometric methods. The assay utilizes synthetic peptide substrate IETD-AFC (AFC, 7-amino-4-trifluoromethyl coumarin). Active caspase 8 cleaves the synthetic substrate to release free AFC which can then be quantified by fluorometry. Compounds to be screened can directly be added to the reaction and the level of inhibition of caspase 8 activity can be determined by comparison of the fluorescence intensity in samples with and without the testing inhibitors.

# 2. Protocol Summary

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### 3. Components and Storage

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#### A. Kit Components

| Item                                | Quantity    |
|-------------------------------------|-------------|
| 2X Reaction Buffer                  | 10 mL       |
| Caspase Substrate IETD-AFC (1 mM)   | 0.5 mL      |
| DTT (1 M)                           | 100 $\mu$ L |
| Active Caspase 8 (Lyophilized)      | 100 units   |
| Caspase Inhibitor, Z-VAD-FMK (2 mM) | 10 $\mu$ L  |

\* Store kit at  $-20^{\circ}\text{C}$ . All components are stable for 6 months under proper storage conditions.

Protect IETD-AFC from light.

**REACTION BUFFER:** After thawing, store the 2X Reaction Buffer at  $+4^{\circ}\text{C}$ . Aliquot enough 2X Reaction Buffer for the number of assays to be performed. Add DTT to the 2X Reaction Buffer immediately before use (10 mM final concentration: add 10  $\mu$ L of 1.0 M DTT stock per 1 mL of 2X Reaction Buffer).

**ACTIVE CASPASE 8:** Reconstitute in 550  $\mu$ L 2X Reaction Buffer. Aliquot and immediately store at  $-80^{\circ}\text{C}$ .

## **B. Additional Materials Required**

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader
- 96-well plate
- Orbital shaker

## 4. Assay Protocol

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1. Prepare test samples in dH<sub>2</sub>O up to a final volume of 50  $\mu$ L/well. Add 5  $\mu$ L of Active Caspase 8. Mix well.

Prepare a **background control** by omitting the Active Caspase 8 from the reaction mixture.

Prepare a **positive inhibition control** by adding 1  $\mu$ L of the Caspase 8 Inhibitor (provided with the kit) instead of your testing inhibitor.

**Note:**

This product detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay.

2. Prepare a Master Mix for each assay containing the following:

|                                  |            |
|----------------------------------|------------|
|                                  | 45 $\mu$ L |
| 2X Reaction Buffer               |            |
| (containing 10 mM DTT)           |            |
| 1 mM IETD-AFC substrate          | 5 $\mu$ L  |
| (50 $\mu$ M final concentration) |            |

3. Mix well and add 50  $\mu$ L of the Master Mix to each well to start the reaction. Incubate at 37°C for 0.5-1 hour.

4. Read samples in a fluorescence plate reader equipped with a 400-nm excitation filter and 505-nm emission filter.

Compare the fluorescence intensity of the test samples with samples containing no inhibitors to determine the inhibition efficiency of the testing inhibitors.

## 5. Factors to consider when assaying caspases

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Three major factors need to be taken into account when using caspase activity assays:

1. The substrate in a particular assay is not necessarily specific to a particular caspase.

Cleavage specificities overlap so reliance on a single substrate/assay is not recommended. Other assays, such as Western blot or use of fluorescent substrates e.g. FRET assays should be used in combination with caspase activity assays.

2. The expression and abundance of each caspase in a particular cell type and cell line will vary.
3. As the activation and cleavage of caspases in the cascade will change over time, you should consider when particular caspase will be at its peak concentration e.g. after 3 hours, after 20 hours etc.

The table below shows the known cross-reactivities with other caspases.

Classification of caspases based on synthetic substrate preference, does not reflect the real caspase substrate preference *in vivo* and may provide inaccurate information for discriminating amongst



caspase activities. Thus, caution is advised in applying the intrinsic tetrapeptide preferences to predict the targets of individual caspases.

### Apoptotic Initiator Caspases

| Caspase           | Cleavage motif | Inhibitor motif | Cross-reactivity with other caspase: |   |   |   |   |   |   |   |    |    |  |   |
|-------------------|----------------|-----------------|--------------------------------------|---|---|---|---|---|---|---|----|----|--|---|
|                   |                |                 | 1                                    | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9  | 10 |  |   |
| <b>Caspase 2</b>  | VDVAD          |                 |                                      |   | Y |   |   |   |   | Y |    |    |  |   |
| <b>Caspase 8</b>  | IETD           | IETD,<br>LETD   |                                      |   | Y |   |   | Y |   |   |    |    |  | Y |
| <b>Caspase 9</b>  | LEHD           |                 |                                      |   | Y |   |   | Y |   | Y |    |    |  | Y |
| <b>Caspase 10</b> | AEVD           |                 |                                      |   | Y |   |   |   |   | Y | Y? |    |  |   |

## 6. Troubleshooting

| <b>Problem</b>     | <b>Reason</b>                         | <b>Solution</b>   |
|--------------------|---------------------------------------|---|
| Assay not working  | Reaction buffer at wrong temperature  | Assay buffer must be at 4°C   |
|                    | Protocol step missed                  | Re-read and follow the protocol exactly   |
|                    | Plate read at incorrect wavelength    | Ensure you are using appropriate reader and filter settings (refer to datasheet)  |
|                    | Unsuitable microtiter plate for assay | Fluorescence: Black plates (clear bottoms);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>If critical, datasheet will indicate whether to use flat- or U-shaped wells |
| Unexpected results | Measured at wrong wavelength          | Use appropriate reader and filter settings described in datasheet   |
|                    | Samples contain impeding substances   | Troubleshoot and also consider deproteinizing samples   |
|                    | Unsuitable sample type                | Use recommended samples types as listed on the datasheet  |

| <b>Problem</b> | <b>Reason</b>                               | <b>Solution</b>   |
|----------------|---|---|
| General Issues | Improperly thawed components                | Thaw all components completely and mix gently before use                  |
|                | Incorrect incubation times or temperatures  | Refer to datasheet & verify the correct incubation times and temperatures |
|                | Incorrect volumes used                      | Use calibrated pipettes and aliquot correctly                             |
|                | Air bubbles formed in the well/tube         | Pipette gently against the wall of the well/tubes                         |
|                | Substituting reagents from older kits/ lots | Use fresh components from the same kit                                    |

**For further technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “*contact us*” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).**







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