

**ab65614**

# **Caspase 8 (active) FITC Staining Kit**

## Instructions for Use

For the rapid, sensitive and accurate detection of active Caspase 8 in living cells

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

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# 1. Overview

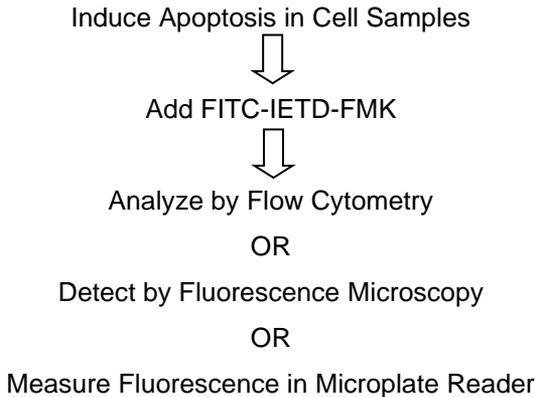
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Activation of caspases plays a central role in apoptosis. Abcam's Caspase 8 (active) FITC Staining Kit provides a convenient means for sensitive detection of activated caspase 8 in living cells.

The assay utilizes the caspase 8 inhibitor, IETD-FMK, conjugated to FITC (FITC-IETD-FMK) as a marker. FITC-IETD-FMK is cell permeable, non-toxic, and irreversibly binds to activated caspase 8 in apoptotic cells. The FITC label allows detection of activated caspase 8 in apoptotic cells directly by fluorescence microscopy, flow cytometry, or fluorescence plate reader.

# 2. Protocol Summary

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### 3. Components and Storage

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#### A. Kit Components

| Item           | Quantity    |
|----------------|-------------|
| FITC-IETD-FMK  | 100 $\mu$ L |
| Wash Buffer IV | 2 x 100 mL  |
| Z-VAD-FMK      | 10 $\mu$ L  |

\* Store kit at -20°C.

#### B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader or microscope
- Flow cytometer
- Black microtiter plate
- Orbital shaker

## 4. Assay Protocol

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### 1. Sample Preparation:

- a) Induce apoptosis in cells ( $1 \times 10^6/\text{ml}$ ) by desired method. Concurrently incubate a control culture *without* induction.

An additional **negative control** can be prepared by adding the caspase inhibitor Z-VAD-FMK at  $1 \mu\text{l}/\text{ml}$  to an induced culture to inhibit caspase 8 activation.

- b) Aliquot  $300 \mu\text{l}$  each of the induced and control cultures into eppendorf tubes.
- c) Add  $1 \mu\text{l}$  of FITC-IETD-FMK into each tube and incubate for 0.5-1 hour at  $37^\circ\text{C}$  incubator with 5%  $\text{CO}_2$ .
- d) Centrifuge cells at 3000 rpm for 5 minutes and remove supernatant.
- e) Re-suspend cells in 0.5 ml of Wash Buffer IV and centrifuge again.
- f) Repeat Step e.
- g) Proceed to Step 2, 3 or 4 depending on methods of analysis.

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

## **2. Quantification by Flow Cytometry:**

For flow cytometric analysis, re-suspend cells in 300  $\mu\text{l}$  of Wash Buffer IV. Keep samples on ice. Analyze samples by flow cytometry using the FL-1 channel.

## **3. Detection by Fluorescence Microscopy:**

For fluorescence microscopic analysis, re-suspend cells in 100  $\mu\text{l}$  Wash Buffer IV. Put one drop of the cell suspension onto a microslide and cover with a coverslip.

Observe cells under a fluorescence microscope using FITC filter. Caspase positive cells appear to have brighter green signals, whereas caspase negative control cells show much weaker signal.

## **4. Analysis by Fluorescence Plate Reader:**

For analysis with fluorescence plate reader, re-suspend cells in 100  $\mu\text{l}$  Wash Buffer IV and then transfer the cell suspension to each well in the black microtiter plate.

Measure the fluorescence intensity at Ex. = 485 nm and Em = 535 nm. For control, use wells containing unlabeled cells.

## 5. Factors to consider for caspase activity assays

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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, 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, 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>   |
|----------------------------|---|---|
| <b>High Background</b>     | Cell density is higher than recommended                 | Refer to datasheet and use the suggested cell number  |
|                            | Increased volumes of components added                   | Use calibrated pipettes accurately  |
|                            | Incubation of cell samples for extended periods         | Refer to datasheets and incubate for exact times  |
|                            | Use of extremely confluent cells                        | Perform assay when cells are at 80-95% confluency   |
|                            | Contaminated cells                                      | Check for bacteria/ yeast/ mycoplasma contamination   |
| <b>Lower signal levels</b> | Cells did not initiate apoptosis                        | Determine the time-point for initiation of apoptosis after induction (time-course experiment) |
|                            | Very few cells used for analysis                        | Refer to data sheet for appropriate cell number   |
|                            | Incorrect setting of the equipment used to read samples | Refer to datasheet and use the recommended filter setting                                     |
|                            | Use of expired kit or improperly stored reagents        | Always check the expiry date and store the components appropriately                           |

| <b>Problem</b>         | <b>Reason</b>                                      | <b>Solution</b>   |
|------------------------|--|---|
| <b>Erratic results</b> | Uneven number of cells seeded in the wells         | Seed only healthy cells (correct passage number)                              |
|                        | Adherent cells dislodged at the time of experiment | Perform experiment gently and in duplicates or triplicates for each treatment |
|                        | Incorrect incubation times or temperatures         | Refer to datasheet & verify correct incubation times and temperatures         |
|                        | Incorrect volumes used                             | Use calibrated pipettes and aliquot correctly                                 |

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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