A.G. Scientific, Inc.
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# Product Data Sheet

**PRODUCT:** Caspase-8 Fluorometric Assay Kit (*BioVision*)

**PRODUCT NUMBER:** C-1415

**LOT NUMBER:** A8602

**INTRODUCTION:**

Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The **Caspase-8 Fluorometric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence IETD. The assay is based on the detection of cleavage of substrate IETD-AFC (AFC: 7-amino-4-trifluoromethylcoumarin). IETD-AFC emits blue light (λmax = 400nm); upon cleavage of the substrate by caspase-8 or related caspases, free AFC emits a yellow-green fluorescence (λmax = 505nm), which can be quantified using a fluorometer or a fluorescence microtiter plate reader. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-8 activity.

**KIT CONTAINS:**

| Components | C-1415<br>25 test |
|---|---|
| Cell Lysis Buffer | 25 ml |
| 2x Reaction Buffer | 2 ml |
| IETD-AFC Substrate (1mM) | 125 µl |
| DTT (1M) | 100 µl |

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## CASPASE-8 ASSAY:

### General Considerations

- Aliquot enough 2x Reaction Buffer for the number of assays to be preformed. Add DTT to the 2x Reaction Buffer immediately before use (10 mM final concentration: add 10 µl of 1.0 M DTT stock per 1 ml of 2x Reaction buffer).
- After thawing, store the Cell Lysis Buffer and 2x Reaction Buffer at +4˚C.
- Protect IETD-AFC from light.

## ASSAY PROCEDURE:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Count cells and pellet 1-5 x $10^6$ cells or use 50-200µg cell lysates if protein concentration has been measured.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer.
4. Incubate cells on ice for 10 minutes.
5. Add 50 µl of 2x Reaction Buffer (containing 10 mM DTT) to each sample.
6. Add 5 µl of the 1 mM IETD-AFC substrate (50 µM final concentration) and incubate at 37˚C for 1-2 hours.
7. Read samples in a fluorometer equipped with a 400nm excitation filter and 505nm emission filter. For plate-reading set-up, transfer the samples to a 96-well plate.

You may also perform the entire assay directly in a 96-well plate.

Fold-increase in Caspase-8 activity can be determined by comparing these results with the level of uninduced control.

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## STORAGE & HANDLING:

Store kit at -20°C (Store Cell Lysis Buffer and 2x Reaction Buffer at +4°C after opening).

All reagents are stable for at least 6 months under proper storage conditions.

CAUTION: For research use only. Not for human or drug use. The pharmacological and toxicological properties of this product have not been fully investigated. Use caution when handling. Do not use this compound if you are not fully trained or are unaware of the hazards involved.

Verified: