



PRODUCT DATA SHEET

PRODUCT: **Caspase-9 Fluorometric Assay Kit**
(BioVision)

PRODUCT NUMBER: C-1417

LOT NUMBER: A8601

INTRODUCTION: Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The **Caspase-9 Fluorometric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence LEHD. The assay is based on the detection of cleavage of substrate LEHD-AFC (AFC: 7-amino-4-trifluoromethylcoumarin). LEHD-AFC emits blue light ($\lambda_{max} = 400nm$); upon cleavage of the substrate by caspase-9 or related caspases, free AFC emits a yellow-green fluorescence ($\lambda_{max} = 505nm$), 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-9 activity.

KIT CONTAINS:	Components	C-1417
		25 test
	Cell Lysis Buffer	25 ml
	2x Reaction Buffer	2 ml
	LEHD-AFC Substrate (1mM)	125 μ l
	DTT (1M)	100 μ l

CASPASE-9 ASSAY:

General Considerations

- 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 μ l of 1.0 M DTT stock per 1 ml of 2x Reaction buffer).
- 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 $1-5 \times 10^6$ cells or use 50-200 μ g cell lysates if protein concentration has been measured.
3. Resuspend cells in 50 μ l of chilled Cell Lysis Buffer.
4. Incubate cells on ice for 10 minutes.
5. Add 50 μ l of 2x Reaction Buffer (containing 10 mM DTT) to each sample.
6. Add 5 μ l of the 1 mM LEHD-AFC substrate (50 μ M final concentration) and incubate at 37°C for 1-2 hours.
7. Read samples in a fluorometer equipped with a 400 nm excitation filter and 505 nm 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: 