



Caspase-8 Fluorometric Assay Kit

Manufactured by BioVision.

ALX-850-222-KI01

~25 tests

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For laboratory use only. Not for human or diagnostic use.

I. 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 detection of cleavage of substrate IETD-AFC (AFC: 7-amino-4-trifluoromethylcoumarin). IETD-AFC emits blue light ($\lambda_{\text{max}} = 400 \text{ nm}$); upon cleavage of the substrate by Caspase-8 or related caspases, free AFC emits a yellow-green fluorescence ($\lambda_{\text{max}} = 505 \text{ nm}$), 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.

II. Kit Contains:

Components	850-222-KI01
	25 tests
Cell Lysis Buffer	25 ml
2x Reaction Buffer	2 ml
IETD-AFC (1 mM)	125 μl
DTT (1 M)	100 μl

III. Caspase-8 Assay Protocol:

A. 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 μl of 1 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.

B. 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 μg cell lysates if protein concentration has been measured.
3. Resuspend cells in 50 μl of chilled Cell Lysis Buffer. Incubate cells on ice for 10 minutes.
4. Add 50 μl of 2x Reaction Buffer (containing 10 mM DTT) to each sample.
5. Add 5 μl of the 1 mM IETD-AFC substrate (50 μM final concentration) and incubate at 37°C for 1-2 hours.
6. Read samples in a fluorometer equipped with a 400 nm excitation filter and 505 nm emission filter. For a 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 the uninduced control.

IV. Storage and Stability:

- 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.