



Caspase-6 Fluorometric Assay Kit

Manufactured by BioVision.

ALX-850-220-KI01

~25 tests

(Version 01: April 1, 2005)

NORTH AMERICA

AXXORA, LLC
6181 Cornerstone Court East
Suite 103
San Diego, CA 92121-4727
Phone: (858) 550-8828
Fax: (858) 550-8825
E-mail: axxora-usa@axxora.com

SWITZERLAND/REST OF THE WORLD

ALEXIS CORPORATION
Industriestrasse 17, Postfach
CH-4415 Lausen / Switzerland
Phone: +41 61 926 89 89
Fax: +41 61 926 89 79
E-mail: alexis-ch@alexis-corp.com

GERMANY

AXXORA DEUTSCHLAND GmbH
Marie-Curie-Strasse 8
DE-79539 Lörrach
Phone: (07621) 5500 522
Fax: (07621) 5500 523
E-mail: axxora-de@axxora.com

UK & IRELAND

AXXORA (UK) LTD.
P.O. Box 6757
Bingham, Nottingham NG13 8LS
Phone: +44 1949 836111
Fax: +44 1949 836222
E-mail: axxora-uk@axxora.com

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-6 Fluorometric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence VEID. The assay is based on detection of cleavage of substrate VEID-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). VEID-AFC emits blue light ($\lambda_{\text{max}} = 400 \text{ nm}$); upon cleavage of the substrate by caspase-6 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-6 activity.

II. Kit Contents:

Components	850-220-KI01
	25 tests
Cell Lysis Buffer	25 ml
2x Reaction Buffer	2 ml
VEID-AFC (1mM)	125 μl
DTT (1M)	100 μl

III. Caspase-6 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 (10mM final concentration: add 10 μl of 1M DTT stock per 1ml of 2x Reaction Buffer).
- After thawing, store Cell Lysis Buffer and 2x Reaction Buffer at 4°C.
- Protect VEID-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 x 10⁶ cells or use 50-250 μg 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 10mM DTT) to each sample.
6. Add 5 μl of the 1mM VEID-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 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-6 activity can be determined by comparing the results of treated samples with the level of the uninduced control.

IV. Storage:

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