



Caspase-2 Colorimetric Assay Kit

Manufactured by BioVision.

ALX-850-213-KI01

~25 tests

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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-2 Colorimetric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence VDVAD. The assay is based on spectrophotometric detection of the chromophore *p*-nitroaniline (*p*NA) after cleavage from the labeled substrate VDVAD-*p*NA. The *p*NA light emission can be quantified using a spectrophotometer or a microtiter plate reader at 400 or 405 nm. Comparison of the absorbance of *p*NA from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-2 activity.

II. Kit Contains:

Components:	850-213-KI01
	25 tests
Cell Lysis Buffer	25 ml
2x Reaction Buffer	2 ml
VDVAD- <i>p</i> NA (4 mM)	125 μ l
DTT (1 M)	100 μ l
Dilution Buffer	25 ml

III. Caspase-2 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 μ l of 1.0 M DTT stock per 1 ml of 2x Reaction Buffer).
- After thawing, store the Cell Lysis Buffer, 2x Reaction Buffer, and Dilution Buffer at +4°C.
- Protect VDVAD-*p*NA 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 2-5 x 10⁶ cells.
3. Resuspend cells in 50 μ l of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes.
4. Centrifuge for 1 minute in a microcentrifuge (10'000 x g).
5. Transfer supernatant (cytosolic extract) to a fresh tube and put on ice.
6. Assay protein concentration.
7. Dilute 100-200 μ g cytosolic extract to 50 μ l Cell Lysis Buffer for each assay.
8. Add 50 μ l of 2x Reaction Buffer (containing 10 mM DTT) to each sample.
9. Add 5 μ l of the 4 mM VDVAD-*p*NA substrate (200 μ M final conc.) and incubate at 37°C for 1-2 hours.
10. Read samples at 400 or 405 nm in a microtiter plate reader, or spectrophotometer using a 100- μ l micro quartz cuvet (Sigma), or dilute sample to 1 ml with Dilution Buffer and using regular cuvet.

Note: Dilution of the samples proportionally decreases the reading.

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

Fold-increase in Caspase-2 activity can be determined by comparing the results of treated samples with the level of the uninduced control.

Note: Background reading from cell lysates and buffers should be subtracted from the readings of both induced and the uninduced samples before calculating fold increase in Caspase-2 activity.

IV. Storage and Stability:

- Store kit at -20°C (Store Cell Lysis Buffer, 2x Reaction Buffer, and Dilution Buffer at 4°C after opening).
- All reagents are stable for at least 4 months under proper storage conditions.