



## **Caspase-6 Colorimetric Assay Kit**

*Manufactured by BioVision.*

**ALX-850-219-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-6 Colorimetric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence VEID. The assay is based on spectrophotometric detection of the chromophore *p*-nitroanilide (*p*NA) after cleavage from the labeled substrate VEID-*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-6 activity.

## II. Kit Contains:

Components	850-219-KI01
	25 tests
Cell Lysis Buffer	25 ml
2x Reaction Buffer	2 ml
VEID- <i>p</i> NA (4 mM)	125 $\mu$ l
DTT (1 M)	100 $\mu$ l
Dilution Buffer	25 ml

## 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 (10 mM final concentration: add 10  $\mu$ l of 1 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 VEID-*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 10<sup>6</sup> cells.
3. Resuspend cells in 50  $\mu$ l of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes.
4. Centrifuge for 1 min 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 100-250  $\mu$ g protein to 50  $\mu$ l Cell Lysis Buffer for each assay.
8. Add 50  $\mu$ l of 2X Reaction Buffer (containing 10 mM DTT) to each sample.
9. Add 5  $\mu$ l of the 4 mM VEID-*p*NA substrate (200  $\mu$ M final conc.) and incubate at +37°C for 1-2 hours (or longer if desired).

10. Read samples at 400 or 405 nm in a microtiter plate reader, or spectrophotometer using a 100- $\mu$ 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-6 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-6 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.