



## **Caspase-5 Colorimetric Assay Kit**

*Manufactured by BioVision.*

**ALX-850-217-KI01**

~25 tests

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

## I. Introduction:

The **Caspase-5 Colorimetric Assay Kit** provides a simple and convenient means for assaying the activity of caspase-5 and related caspases that recognize the sequence WEHD. The assay is based on spectrophotometric detection of the chromophore *p*-nitroaniline (*p*NA) after cleavage from the labeled substrate WEHD-*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 a treated sample with an untreated control allows determination of the fold increase in Caspase-5 activity.

## II. Kit Contents:

Components	850-217-KI01
	25 tests
Cell Lysis Buffer	25 ml
2x Reaction Buffer	2 ml
WEHD- <i>p</i> NA (4 mM)	125 $\mu$ l
DTT (1 M)	100 $\mu$ l
Dilution Buffer	25 ml

## III. Caspase-5 Assay Protocol:

### A. Reagent Preparation

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).

### B. Assay Procedure

1. Induce apoptosis by desired method. Concurrently incubate a control culture *without* treatment.
2. Pellet  $2-5 \times 10^6$  cells.
3. Resuspend in 50  $\mu$ l of chilled Cell Lysis Buffer and incubate on ice for 10 min.
4. Centrifuge for 1 min in a microcentrifuge (10'000 x g).
5. Transfer supernatant (cytosolic extract) to a fresh tube and then keep on ice.
6. Assay protein concentration.
7. Dilute 100-200  $\mu$ g protein to 50  $\mu$ l Cell Lysis Buffer for each assay.
8. Add 50  $\mu$ l 2x Reaction Buffer (containing 10 mM DTT) to each sample.
9. Add 5  $\mu$ l of the 4 mM WEHD-*p*NA substrate (200  $\mu$ M final conc.). Incubate at 37°C for 1-2 hour.
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 use regular cuvet (note: Dilution of the samples proportionally decreases the reading).  
You may also perform the assay in a 96-well plate.

Fold-increase in Caspase-5 activity can be determined by comparing the results of treated samples with the level of the untreated control.

**Note:** Background reading from cell lysates and buffers should be subtracted from the readings of both treated and the untreated samples before calculating fold increase in Caspase activity.

#### **IV. Storage and Stability:**

- Store kit at  $-20^{\circ}\text{C}$
- Store Cell Lysis Buffer, 2x Reaction Buffer, and Dilution Buffer at  $4^{\circ}\text{C}$  after opening.
- Protect WEHD-pNA from light.