



Caspase-1 Colorimetric Assay Kit

Manufactured by BioVision.

ALX-850-211-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-1 Colorimetric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence YVAD. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate YVAD-pNA. The pNA light emission can be quantified using a spectrophotometer or a microtiter plate reader at 400 or 405 nm. Comparison of the absorbance of pNA from an apoptotic sample with an uninduced control allows determination of the fold increase in caspase-1 activity.

II. Kit Contains (25 tests):

Components:	850-213-KI01 25 tests
Cell Lysis Buffer	25 ml
2x Reaction Buffer	2 ml
YVAD-pNA Substrate (4 mM)	125 µl
DTT (1 M)	100 µl
Dilution Buffer	25 ml

III. Caspase-1 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 1.0 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 YVAD-pNA from light.

B. Assay Procedure

1. Induce apoptosis or treat cells by desired method. Concurrently incubate a control culture *without* treatment.
Note: Caspase-1 (active) (human) (recombinant) (Prod. no. ALX-201-056) can be used as a positive control for caspase-1 activity assays.
2. Pellet $2-5 \times 10^6$ cells or use 100-200 µg cell lysates if protein concentration has been measured.
3. Resuspend in 50 µl of chilled Cell Lysis Buffer and incubate 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 keep on ice.
6. Assay protein concentration.
7. Dilute 100-200 µg protein to 50 µl Cell Lysis Buffer for each assay.
8. Add 50 µl of 2x Reaction Buffer (containing 10 mM DTT) to each sample.
9. Add 5 µl of the 4 mM YVAD-pNA substrate (200 µM final concentration) 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-1 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-1 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 6 months under proper storage conditions.