# Caspase-1 Colorimetric Assay Kit Catalog TBS2040

100 assays; store at -20℃

## DESCRIPTION

Apoptosis plays a fundamental role in many normal biological processes as well as in several disease states. Apoptosis can be induced by various stimuli that all produce the same end result: systematic and orderly cell death.

The inflammasome is a large multiprotein complex whose assembly leads to the activation of caspase-1, which promotes the maturation of proinflammatory cytokines: interleukin-1β (IL-1β) and IL-18.

The Tribo™ Caspase-1 Colorimetric Assay Kits provide 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 the determination of the fold increase in caspase-1 activity.

## Kit Components and Storage for 100 Assays

| Name | 100 Assays | Store |
|---|---|---|
| Cell Lysis Buffer-A | 6 mL | 4℃ |
| Assay Buffer-B | 6 mL | 4℃ |
| Substrate | 120 µL | -20℃ |
| DTT (1M) | 0.6 mL | -20℃ |
| Shelf Life: 1year. | | |

## APPLICATIONS

- Apoptosis
- Drug screening
- Growth factors
- Cytotoxicity

## DIRECTIONS FOR USE

1. Treatment cells by desired method include without induction control. We recommend performing another two control reactions: (1) apoptosis inducer positive control; (2) caspase-1 inhibitor treated induced cells control.
2. Count cells and pellet 2-5x$10^6$ cells in 1.5 mL tubes.
3. Resuspend ells in 50 µL of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes.
4. Centrifuge for 1 min (10,000 x g).
5. Transfer supernatant (cytosolic extract) to a fresh tube and put on ice for immediate assay or aliquot and store at –80°C for future use.
6. Measure protein concentration (Protein assay kit, TBS2005).
7. At 96 wells flat clear plate, add 50-200 µg sample protein into 50 µL Cell Lysis Buffer for each assay.
8. Immediately before use, prepare enough working reagent by per assay add 50 µL Assay buffer, 5 µL DTT, 1 µL substrate.
9. Transfer 50µL working reagent into sample wells.
10. Seal plate with plate sealer. Incubate at 37°C for 1-2hr, protect from light.
11. Read plate at 405nm in a plate reader, or spectrophotometer using a 100-µl micro quartz cuvette, or use 1cm cuvette by add 700µL PBS.
12. Fold-increase in Caspase-1 activity can be determined by comparing these results 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-1 activity.

The following materials are required but not supplied:

- Caspase-1 inhibitor;
- Apoptosis inducer;
- 96-well clear flat plate or reaction tubes;
- Plate reader or Spectrophotometer.

## RELATED PRODUCTS

Resazurin Cell Viability (TBS2001)
LDH Cytotoxicity Assay (TBS2002)
MTT Cell Viability Assay (TBS 2003)
MTS Cell Viability Assay (TBS2004)
Catalase Assay (TBS2006)
ATP Colorimetric/ Fluorometric Assay (TBS 2010)
ADP Colorimetric/Fluorometric Assay (TBS3020)
XTT Cell Viability Assay (TBS2021)
Caspase-3 Colorimetric Assay (TBS2030)
Caspase Family Colorimetric Assay (TBS2050)
BrdU Cell Proliferation Colorimetric Assay (TBS2086)
Cytochrome c Reductase Activity Assay (TBS2116)
AOPI Viability Assay for Flow Cytometry (TBS2069)

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