

## 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 result: systematic and orderly cell death.

Caspases cleave a variety of cellular substrates after aspartic acid residues - a characteristic that is central to their role in mammalian apoptosis. Caspases are synthesized in the cytosol of mammalian cells as inactive zymogens, which become active through intracellular caspase cascades.

The Tribio™ Caspase-3 fluorometric Assay Kits provide a simple and convenient method for the activity analysis of caspases that recognize the sequence DEVD. The assay is based on the detection of the free AFC fluorescence after cleavage from the labeled substrate DEVD-AFC. The free AFC can be quantified using a fluorometer or a fluorescence microtiter plate reader at Ex400/Em505nm. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows the determination of the fold increase in caspase-3 activity.

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 Ex400/Em505nm in a fluorescence plate reader.
12. Fold-increase in Caspase-3 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-3 activity.

The following materials are required but not supplied:

- Caspase-3 inhibitor.
- Apoptosis inducer.
- 96-well clear flat plate or reaction tubes.
- Plate reader or Spectrophotometer.

## Kit Components and Storage for 100 Assays

Name	100 Assays	Store
Cell Lysis Buffer-A	6 mL	4°C
Assay Buffer-B	6 mL	4°C
Substrate	120 µL	-20°C
DTT (1M)	0.3 mL	-20°C
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 2-5x10<sup>6</sup> cells in 1.5 mL tubes.
3. Resuspend cells 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.

## 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-1 Colorimetric Assay (TBS2040)  
 Caspase-1 Fluorometric Assay (TBS2045)  
 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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