Caspase-3 Colorimetric Assay Kit

(Catalog #TBS2030; 100 assays; store at -20°C)

INTRODUCTION

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.

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 Tribo™ Caspase-3 Colormetric Assay Kits provides a simple and convenient means for assaying the activity of caspases that recognize the sequence DEVD. The assay is based on spectrophotometric detection of the chromophore *P*-nitroaniline (*P*NA) after cleavage from the labeled substrate DEVD-*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-3 activity.

KIT CONTENTS

Cell Lysis Buffer 20 mL Assay Buffer 6 mL
Substrate 120 ∝L 4 mM DTT 240 ∝L 1M

STORAGE AND HANDLING

Store Cell Lysis buffer and Assay buffer at 4°C, Store all other components at -20°C. Shelf life of six months.

ASSAY PROTOCOL

1. Treatment cells by desired method include without induction control. We recommend performing another two control reactions (1) apoptosis inducer positive control; (2) caspase-3 inhibitor treated induced cells control.
2. Count cells and pellet 2-5x10^6 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.
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 700 µL PBS.
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

Reference

1. Thornberry, N.A. & Littlewood, Y. (1998) Caspases: Enemies Within. Science **281:**1312–1316
2. Casciola-Rosen, L. A., Nicholson, D. W., Chong, K. R., Rowan, K. R., Thornberry, N. A., Miller, D. K. & Rosen, A. (1996) Apopain/CPP32 cleaves proteins that are essential for cellular repair: a fundamental principle of apoptotic cell death. J. Exp. Med. **183**:1957–1964
3. Zou, H., Li, Y., Liu, X. & Wang, X. (1999) An APAF-1 cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J. Biol. Chem. **274**:11549–11556.

RELATED PRODUCTS:

Cell Viability Assay Kit (#TBS2001)

ATP Colorimetric/Fluorometric Assay Kit (#TBS2010)

ADP Colorimetric/Fluorometric Assay Kit (#TBS2020)

ADP/ATP Ratio Assay Kit (#TBS2015)

Protein Assay Kits (#TBS2005)

Caspase-3 Fluorometric Assay Kit (#TBS2035)

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