Caspase-8 Colorimetric Assay Kit Cat. No. L00303

Technical Manual No. 0273 **Version 02202008**

I. DESCRIPTION

The Caspase-8 Colorimetric Assay Kit (Cat. No. L00303) provides a simple and convenient means of assaying the activity of caspase-8. The activation of ICE-family proteases and caspases initiates apoptosis in mammalian cells. The assay for caspase-8 is based on spectrophotometric detection of the chromophore *p*nitroanilide (*p*NA) after cleavage from the labeled substrate IETD-*p*NA. The *p*NA light emission can be quantified using a spectrophotometer or a microtiter plate reader at 400 or 405 nm. By comparing the absorbance of *p*NA from an apoptotic sample to an uninduced control, the relative increase of caspase-8 activity can be determined.

II. KEY FEATURES

- **Simplified Procedure:** The components in this kit are provided ready-to-use.
- **Supreme Reliability:** The kit produces highly reproducible results.

III. KIT CONTENTS

The Caspase-8 Colorimetric Assay Kit is available (L00303) employs a substrate IETD-*p*NA. Each kit contains enough reagents for one hundred apoptosis assays.

Components	Cat. No. L00303 100 assays
Lysis Buffer	15.0 ml
2 Reaction Buffer	5.0 ml
Caspase-8 Substrate	500 µl, store away from light
DTT	150 µl

IV. STORAGE

Store the Lysis Buffer and 2X Reaction Buffer at 4°C after opening. Store the rest of the kit at -20°C. All reagents will remain stable for one year.

V. CASPASE-8 COLORIMETRIC ASSAY KIT PROTOCOL

A. General Considerations

- Aliquot enough 2X Reaction Buffer for the number of assays to be performed. Add DTT into the 2X Reaction Buffer immediately to a final concentration of 10 mM before use (10 µl of 1.0 M DTT stock in per 1 ml 2X Reaction Buffer).
- After thawing, store the Lysis Buffer at 4°C.
- Do not expose the Caspase-8 Substrate to light.

B. Assay Procedure

1. Induce apoptosis and set a negative co
2. ntrol at the same time.
3. Collect the cells and wash them twice with PBS by centrifugation at 2000 rpm for five minutes.
4. Collect 3×10^6-5×10^6 cells and try to remove the PBS.
5. Add 50 µl cold prepared Lysis Buffer into the collected cells and mix well. (Note: Before using the Lysis Buffer, add 0.5 µl DTT to 50 µl Lysis Buffer.)
6. Incubate the cells on ice for 20-60 minutes, and vortex with vibration three or four times for 10 seconds each time or freeze and thaw the cells two or three times.
7. Centrifuge at 10,000 rpm at 4°C for one minute.
8. Remove the supernatant and transfer it to a new tube. Put the tube on ice.
9. Obtain a small quantity of supernatant to assay the protein concentration by the Bradford or BCA method.
10. Take 50 µl supernatant containing 100-200 µg protein. If there is not enough volume, add Lysis Buffer.
11. Add 50 µl of 2X Reaction Buffer to each sample. (Note: Add 0.5 µl DTT to 50 µl 2X Reaction Buffer.)
12. Add 5 µl Caspase-8 Substrate (200 µM final concentration) and incubate at 37°C for four hours, away from light.
13. Determine the extinction value of the samples using a spectrophotometer (100 µl micro quartz cuvet) or a microtiter plate reader at 400 nm or 405 nm. Obtain the results of the induced group's caspase-8 activity by computing $OD_{inducer}/OD_{negtive\ control}$.

Note: The background from cell lysates and buffers from the readings of induced samples and negative controls should be subtracted before calculating the increase in caspase-8 activity.

VI. RELATED PRODUCTS

Caspase-3 Colorimetric Assay Kit, Cat. No. L00289
Caspase-9 Colorimetric Assay Kit, Cat. No. L00304
Caspase-2 Colorimetric Assay Kit, Cat. No. L00294
Caspase-6 Colorimetric Assay Kit, Cat. No. L00302

VII. ORDERING INFORMATION

Caspase-8 Colorimetric Assay Kit, Cat. No. L00303

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