# Product Information

## Caspase Colorimetric Substrate Set II Plus

### I. Kit Contents:

| Description | Concentration | 25 assays |
|---|---|---|
| Caspase-1 Substrate, Ac-YVAD-pNA | 4 mM | 125 µl |
| Caspase-2 Substrate, Ac-VDVAD-pNA | 4 mM | 125 µl |
| Caspase-3 Substrate, Ac-DEVD-pNA | 4 mM | 125 µl |
| Caspase-4 Substrate, Ac-LEVD-pNA | 4 mM | 125 µl |
| Caspase-5 Substrate, Ac-WEHD-pNA | 4 mM | 125 µl |
| Caspase-6 Substrate, Ac-VEID-pNA | 4 mM | 125 µl |
| Caspase-8 Substrate, Ac-IETD-pNA | 4 mM | 125 µl |
| Caspase-9 Substrate, Ac-LEHD-pNA | 4 mM | 125 µl |
| Caspase-10 Substrate, Ac-AEVD-pNA | 4 mM | 125 µl |
| Cell Lysis Buffer | N/A | 100 ml |
| Dilution Buffer | N/A | 200 ml |
| 2X Reaction Buffer | N/A | 20 ml |
| DTT | 1 M | 0.4 ml |

### II. Introduction:

Caspases (Cysteine-dependent aspartate-directed proteases) are a family of cysteine proteases that play important roles in apoptosis, inflammation and necrosis. Sequential activation of caspases plays a critical role in cell apoptosis. Caspase-1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 are members of caspases family. Caspase-1, 4 and 5 are involved in T-cell maturation. Caspase-2, 8, 9 and 10 are initiator caspases that cleave inactive pro-forms of effector caspases into active caspases. Caspase-3, 6 and 7 are effector caspases that cleave other protein substrates within the cell to trigger the apoptotic process.

Caspase Colorimetric Substrate Set II Plus is composed of ready-to-use pNA-labeled substrates for Caspase-1, 2, 3, 4, 5, 6, 8, 9 and 10 of caspase family proteases. The kit is used to detect activities of members of caspase family proteases. All substrates are provided in liquid form.

### III. Assay Procedure:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Count cells and pellet 1 - 5 x 106 cells.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes. Centrifuge for 1 min in a microcentrifuge (10,000 x g).
5. Transfer supernatant to a fresh tube and assay protein Concentration.
6. Dilute 100 - 300 µg protein to 50 µl Cell Lysis Buffer for each assay.
7. Add 50 µl of 2X Reaction Buffer containing 10 mM DTT to each sample.
8. Add 5 µl of the 4 mM pNA conjugated substrates (200 µM final conc.) into each tube individually and incubate at 37℃ for 1 - 2 hour.

9 Read samples at 400 or 405 nm in a microtiter plate reader, or spectrophotometer using a 100 µl micro quartz cuvette, or dilute sample to 1 ml with Dilution Buffer and using regular cuvette (note: Dilution of the samples proportionally decreases the reading).

Fold-increase in caspase activity can be determined by comparing these results with the level of the uninduced control.

Note: Background reading from cell lysates and buffers must be subtracted from the readings of both induced and the uninduced samples before you calculate the fold increase in caspase activity.

**General Troubleshooting Guide for Caspase Colorimetric and Fluorometric Kits:**

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Cells did not lyse completely<br>• Experiment was not performed at optimal time after apoptosis induction<br>• Plate read at incorrect wavelength<br>• Old DTT used | • Resuspend the cell pellet in the lysis buffer and incubate as described in the datasheet<br>• Perform a time-course induction experiment for apoptosis<br>• Check the wavelength listed in the datasheet and the filter settings of the instrument<br>• Always use freshly thawed DTT in the cell lysis buffer |
| High Background | • Increased amount of cell lysate used<br>• Increased amounts of components added due to incorrect pipetting<br>• Incubation of cell samples for extended periods<br>• Use of expired kit or improperly stored reagents<br>• Contaminated cells | • Refer to datasheet and use the suggested cell number to prepare lysates<br>• Use calibrated pipettes<br>• Refer to datasheet and incubate for exact times<br>• Always check the expiry date and store the individual components appropriately<br>• Check for bacteria/ yeast/ mycoplasma contamination |
| Lower signal levels | • Cells did not initiate apoptosis<br>• Very few cells used for analysis<br>• Use of samples stored for a long time<br>• Incorrect setting of the equipment used to read samples<br>• Allowing the reagents to sit for extended times on ice | • Determine the time-point for initiation of apoptosis after induction (time-course experiment)<br>• Refer to datasheet for appropriate cell number<br>• Use fresh samples or aliquot and store and use within one month for the assay<br>• Refer to datasheet and use the recommended filter setting<br>• Always thaw and prepare fresh reaction mix before use |
| Samples with erratic readings | • Uneven number of cells seeded in the wells<br>• Samples prepared in a different buffer<br>• Adherent cells dislodged and lost at the time of experiment<br>• Cell/ tissue samples were not completely homogenized<br>• Samples used after multiple freeze-thaw cycles<br>• Presence of interfering substance in the sample<br>• Use of old or inappropriately stored samples | • Seed only equal number of healthy cells (correct passage number)<br>• Use the cell lysis buffer provided in the kit<br>• Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters<br>• Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope<br>• Aliquot and freeze samples, if needed to use multiple times<br>• Troubleshoot as needed<br>• Use fresh samples or store at correct temperatures until use |
| Unanticipated results | • Measured at incorrect wavelength<br>• Cell samples contain interfering substances | • Check the equipment and the filter setting<br>• Troubleshoot if it interferes with the kit (run proper controls) |
| General issues | • Improperly thawed components<br>• Incorrect incubation times or temperatures<br>• Incorrect volumes used<br>• Air bubbles formed in the well/tube | • Thaw all components completely and mix gently before use<br>• Refer to datasheet & verify the correct incubation times and temperatures<br>• Use calibrated pipettes and aliquot correctly |

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|---|---|---|
| | • Substituting reagents from older kits/ lots<br>• Use of a different 96-well plate | • Pipette gently against the wall of the well/tubes<br>• Use fresh components from the same kit<br>• Fluorescence: Black plates; Absorbance: Clear plates |
| Note# The most probable cause is listed under each section. Causes may overlap with other sections. | | |

**For research use only! Not to be used in humans.**

## Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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