

## Product Information

### Caspase-3 Fluorometric Assay Kit

#### I. Kit Contents:

| Component          | K2007-25<br>25 assays | K2007-100<br>100 assays | K2007-200<br>200 assays | K2007-400<br>400 assays | Part Number |
|--------------------|-----------------------|-------------------------|-------------------------|-------------------------|-------------|
| Cell Lysis Buffer  | 25 ml                 | 100 ml                  | 100 ml                  | 100 ml                  | K2007-C-1   |
| 2X Reaction Buffer | 2 ml                  | 4 x 2 ml                | 16 ml                   | 32 ml                   | K2007-C-2   |
| DEVD-AFC (1 mM)    | 125 $\mu$ l           | 500 $\mu$ l             | 2 x 0.5 ml              | 2 x 1 ml                | K2007-C-3   |
| DTT (1 M)          | 100 $\mu$ l           | 400 $\mu$ l             | 400 $\mu$ l             | 400 $\mu$ l             | K2007-C-4   |

#### II. Introduction:

Cysteine-dependent aspartate-directed proteases (Caspases) are a family of cysteine proteases that play important roles in apoptosis, necrosis, and inflammation. Sequential activation of caspases plays an important role in cell apoptosis. Caspase-3 is a caspase protein that cleaves and activates caspases-6 and -7, and is processed and activated by caspases-8,-9, and-10. Caspase-3 is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor protein, which is associated with Alzheimer's disease. Caspase-3 recognizes tetra-peptide sequences D-x-x-D and hydrolyzes peptide bonds after aspartic acid residues.

Caspase-3 Fluorometric Assay Kit provides a convenient and simple way for detecting the DEVD-dependent caspase activity. DEVD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin) emits blue light ( $\lambda_{max} = 400$  nm); while cleavage of DEVD-AFC by Caspase-3 or related caspases, free AFC emits a yellow-green fluorescence ( $\lambda_{max} = 505$  nm), which can be quantified by using a fluorescence microtiter plate reader or a fluorometer. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control determines the fold increase in Caspase-3 activity.

#### III. Caspase-3 Assay Protocol:

##### A. General Considerations

Aliquot enough 2X Reaction Buffer for the number of assays to be performed. Add DTT to the 2X Reaction Buffer immediately before use (10 mM final concentration: add 10  $\mu$ l of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer).

After thawing, store the Cell Lysis Buffer and 2X Reaction Buffer at 4°C.

Protect DEVD-AFC from light.

##### B. Assay Procedure

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Count cells and pellet  $1-5 \times 10^6$  cells or use 20-200  $\mu$ g cell lysates (depending on the apoptosis level).
3. Resuspend cells in 50  $\mu$ l of chilled Cell Lysis Buffer.
4. Incubate cells on ice for 10 minutes.
5. Add 50  $\mu$ l of 2X Reaction Buffer (containing 10 mM DTT) to each sample.
6. Add 5  $\mu$ l of the 1 mM DEVD-AFC substrate (50  $\mu$ M final concentration) and incubate at 37°C for 1-2 hour.
7. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. You may also perform the entire assay directly in a 96-well plate.

#### Notes:

1) For tissue samples, tissue can be homogenized in Lysis Buffer (for 1X volume of tissue, add 3X volume of lysis buffer) to generate tissue lysate, then follow the kit procedure.

2) Tissue and cell lysates can be kept frozen at -80°C for up to 2 months without significant loss of activity.

### General Troubleshooting Guide Caspase Kits:

| Problems                      | Cause  | Solution  |
|-------------------------------|--|---|
| Assay not working             | <ul style="list-style-type: none"> <li>• Cells did not lyse completely</li> <li>• Experiment was not performed at optimal time after apoptosis induction</li> <li>• Plate read at incorrect wavelength</li> <li>• Old DTT used</li> </ul>  | <ul style="list-style-type: none"> <li>• Resuspend the cell pellet in the lysis buffer and incubate as described in the datasheet</li> <li>• Perform a time-course induction experiment for apoptosis</li> <li>• Check the wavelength listed in the datasheet and the filter settings of the instrument</li> <li>• Always use freshly thawed DTT in the cell lysis buffer</li> </ul>  |
| High Background               | <ul style="list-style-type: none"> <li>• Increased amount of cell lysate used</li> <li>• Increased amounts of components added due to incorrect pipetting</li> <li>• Incubation of cell samples for extended periods</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Contaminated cells</li> </ul>  | <ul style="list-style-type: none"> <li>• Refer to datasheet and use the suggested cell number to prepare lysates</li> <li>• Use calibrated pipettes</li> <li>• Refer to datasheet and incubate for exact times</li> <li>• Always check the expiry date and store the individual components appropriately</li> <li>• Check for bacteria/ yeast/ mycoplasma contamination</li> </ul>  |
| Lower signal levels           | <ul style="list-style-type: none"> <li>• Cells did not initiate apoptosis</li> <li>• Very few cells used for analysis</li> <li>• Use of samples stored for a long time</li> <li>• Incorrect setting of the equipment used to read samples</li> <li>• Allowing the reagents to sit for extended times on ice</li> </ul>   | <ul style="list-style-type: none"> <li>• Determine the time-point for initiation of apoptosis after induction (time-course experiment)</li> <li>• Refer to datasheet for appropriate cell number</li> <li>• Use fresh samples or aliquot and store and use within one month for the assay</li> <li>• Refer to datasheet and use the recommended filter setting</li> <li>• Always thaw and prepare fresh reaction mix before use</li> </ul>  |
| Samples with erratic readings | <ul style="list-style-type: none"> <li>• Uneven number of cells seeded in the wells</li> <li>• Samples prepared in a different buffer</li> <li>• Adherent cells dislodged and lost at the time of experiment</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple freeze-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul> | <ul style="list-style-type: none"> <li>• Seed only equal number of healthy cells (correct passage number)</li> <li>• Use the cell lysis buffer provided in the kit</li> <li>• Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope</li> <li>• Aliquot and freeze samples, if needed to use multiple times</li> <li>• Troubleshoot as needed</li> <li>• Use fresh samples or store at correct temperatures until use</li> </ul> |
| Unanticipated results         | <ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Cell samples contain interfering substances</li> </ul>  | <ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit (run proper controls)</li> </ul>  |
| General issues                | <ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>   | <ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Refer to datasheet &amp; verify the correct incubation times and temperatures</li> </ul>   |

|   |  |   |
|---|--|---|
|   | <ul style="list-style-type: none"><li>• Air bubbles formed in the well/tube</li><li>• Substituting reagents from older kits/ lots</li><li>• Use of a different 96-well plate</li></ul> | <ul style="list-style-type: none"><li>• Use calibrated pipettes and aliquot correctly</li><li>• Pipette gently against the wall of the well/tubes</li><li>• Use fresh components from the same kit</li><li>• Fluorescence: Black plates; Absorbance: Clear plates</li></ul> |
| 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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