

Product name: Caspase-2 Fluorometric Assay Kit

Packing: 25 assays./ 100 assays/200 assays/400 assays

Catalog number:CM261767

I. Introduction:

Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The **Caspase-2/ICH-1 Fluorometric Protease Assay Kits** provide a simple and convenient means for assaying the activity of caspases that recognize the sequence VDVAD. The assay is based on detection of cleavage of substrate VDVAD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). VDVAD-AFC emits blue light ($\lambda_{\max} = 400$ nm); upon cleavage of the substrate by ICH-1 or related caspases, free AFC emits a yellow-green fluorescence ($\lambda_{\max} = 505$ nm), which can be quantified using a fluorometer or a fluorescence microtiter plate reader. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-2 activity.

II. Kit Contents:

Components	CM261767 25 assays	CM261767 100 assays	CM261767 200assays	CM261767 400 assays
Cell Lysis Buffer	25 ml	100 ml	100 ml	100 ml
2X Reaction Buffer	2 ml	4 x 2 ml	16 ml	32 ml
VDVAD-AFC (1 mM)	125 μ l	0.5 ml	2 x 0.5 ml	2 x 1.0 ml
DTT (1 M)	100 μ l	400 μ l	400 μ l	400 μ l

III. Caspase-2 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 μ 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 VDVAD-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 x 10⁶ cells or use 50-200 μ g cell lysates if protein concentration has been measured.
3. Resuspend cells in 50 μ l of chilled Cell Lysis Buffer.
4. Incubate cells on ice for 10 minutes.
5. Add 50 μ l of 2X Reaction Buffer (containing 10 mM DTT) to each sample.

6. Add 5 μ l of the 1 mM VDVAD-AFC substrate (50 μ 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.

Fold-increase in Caspase-2 activity can be determined by comparing these results with the level of the uninduced control.

IV. Storage and Stability:

- Store kit at -20°C. All reagents are stable for at least 6 months.

VI. Related Products:

1. KinaseSTAR™ JNK & Akt Activity Assay Kits
2. Histone Deacetylase Fluorometric & Colorimetric Assay Kits
3. Calpain Activity Assay Kit
4. ApoSENSOR™ High Throughput Cell Viability Kit & ADP/ATP Ratio Kit
5. MitoCapture™ MitoChondrial Apoptosis Detection Kit
6. Mitochondria/Cytosol Fractionation Kit
7. Cytochrome c Releasing Apoptosis Assay Kit
8. ApoGSH™ Glutathion Detection Kit
9. Nitric Oxide Fluorometric & Colorimetric Detection Kits
10. Annexin V-FITC, -Cy3, -Cy5, -EGFP, -PE, -Biotin Kits & Bulk Reagents
11. CaspGLOW™ Active Caspase Staining Kits
12. CaspSCREEN™ Flow Cytometric Apoptosis Assay Kit
13. CaspSELECT™ Caspase-3 & -7 Immunoassay Kits
14. Caspase-1, -2, -3, -4, -5, -6, -8, -9, -10 Fluorometric and Colorimetric Assay Kits
15. Active Human Caspase-1,-2,-3,-4,-5,-6,-7,-8,-9,-10 & Procaspace-3
16. Anti-Caspase-1,-2,-3,-4,-5,-6,-7,-8,-9,-10,-11,-12,-13,-14 & Active Caspases
17. Anti-Bcl-2, Bad, Bax, Bid, Bim/Bod, Cytochrome c, Cytochrome p450, Mcl-1
18. Anti-DFF40, DFF45, BAFF, p53, Lamin A, Lamin B1, TLR4, RGS3, Survivin
19. Many other Apoptosis Related Antibodies
20. Ready-to-use Caspase Substrates & Sets
21. Ready-to-use Caspase Inhibitors and Sets
22. Quick Apoptotic DNA Ladder Detection Kit
23. Ready-to-use Apoptosis Inducers and Set
24. Quick Cell Proliferation Assay Kit
25. LDH-Cytotoxicity Assay Kit
26. Live-Dead Cell Staining Kit
27. Growth Factors, Cytokines, & Chemokines (Proteins & Antibodies)
28. Anti-GFP Polyclonal Antibody
29. Link-FAST™ 5 Minutes DNA Ligation Kit
30. Gel-FAST™ 20 Minutes Gel Staining/Destaining Kit