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## Technical Bulletin

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# CaspACE™ Assay System, Fluorometric

INSTRUCTIONS FOR USE OF PRODUCT G3540.



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# CaspACE™ Assay System, Fluorometric

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## 1. Description

The CaspACE™ Assay System, Fluorometric, provides reagents to measure the activity of the caspase, or interleukin-1 $\beta$ -converting enzyme (ICE/CED-3), family of cysteine aspartic acid-specific proteases. These proteases play key roles in inflammation and apoptosis in mammalian cells (1-4). The CaspACE™ Assay System, Fluorometric, provides fluorogenic substrates and inhibitors that allow highly sensitive, quantitative measurement of both ICE (caspase-1) and CPP32 (caspase-3) protease activities. The use of the two selective substrates and inhibitors provided allows discrimination between ICE and CPP32 activities. This assay system may be used with purified enzyme preparations or cell extracts and also can be adapted for use in high-throughput systems.

Activation of caspases occurs as a result of growth factor withdrawal, exposure to radiation or chemotherapeutic agents, or initiation of the Fas/Apo-1 receptor-mediated cell death process. Active caspases participate in a cascade of cleavage events, which disable key homeostatic and repair enzymes and bring about systematic structural disassembly of dying cells. The biological substrates of caspases include poly-(ADP ribose) polymerase (PARP), DNA-dependent protein kinase (DNA-PK), lamins, topoisomerases, Gas2, protein kinase C (PKC)  $\delta$ , sterol regulatory element binding proteins (SREBP), U1-70kDa protein and Huntingtin protein (5-8).

Several mammalian homologs of CED-3 (pro-apoptotic gene of *C. elegans*) have been described (9-12). Among these, subfamilies have been identified based on amino acid sequence and substrate and inhibitor specificities. The ICE-like proteases (caspase-1) show specificity for cleavage at the C-terminal side of the aspartate residue of the sequence YVAD (Tyr-Val-Ala-Asp) and are inhibited by the tetrapeptide inhibitor Ac-YVAD-CHO. The CPP32-like proteases (caspase-3) show specificity for cleavage at the C-terminal side of the aspartate residue of the sequence DEVD (Asp-Glu-Val-Asp) and are inhibited by the tetrapeptide inhibitor Ac-DEVD-CHO (2).

## Assay Principle

The fluorogenic substrates for ICE/caspase-1 (Ac-YVAD-AMC) and CPP32/caspase-3 (Ac-DEVD-AMC) provided in the CaspACE™ Assay System are labeled with the fluorochrome 7-amino-4-methyl coumarin (AMC). The substrates produce a blue fluorescence that can be detected by exposure to UV light at 360nm. AMC is released from these substrates upon cleavage by ICE or CPP32-like enzymes. Free AMC produces a yellow-green fluorescence that is monitored by a fluorometer at 460nm. The amount of yellow-green fluorescence produced upon cleavage is proportional to the ICE or CPP32 activity present in the sample (13).

Two potent reversible aldehyde inhibitors of ICE and CPP32 (Ac-YVAD-CHO and Ac-DEVD-CHO, respectively) are provided in the CaspACE™ Assay System. In cell lysates, the fluorogenic substrates may be susceptible to cleavage by other related proteinases; therefore, to assess the specific contribution of ICE or CPP32 enzyme activity in crude cell extracts, assays are performed in the presence and absence of selective inhibitors for ICE or CPP32. The difference between substrate cleavage activities in the presence and absence of inhibitor reflects the contribution of ICE or CPP32 enzyme activity.

### Selected Citations Using the CaspACE™ Assay System, Fluorometric

- Al-Zoubi, A.M. *et al.* (2001) Contrasting effects of IG20 and its splice isoforms, MADD and DENN-SV, on tumor necrosis factor  $\alpha$ -induced apoptosis and activation of Caspase-8 and -3. *J. Biol. Chem.* **276**, 47202–11.

This study examined the activation of specific caspases in response to tumor necrosis factor  $\alpha$  in HeLa cells stably transfected with the IG20 cDNA or its splice isoforms using the CaspACE™ Assay System.

- McGinty, A. *et al.* (2000) Cyclooxygenase-2 expression inhibits trophic withdrawal apoptosis in nerve growth factor-differentiated PC12 cells. *J. Biol. Chem.* **275**, 12095-101.

PC12 cells were transfected to express IPTG-inducible cyclooxygenase-2 (Cox-2). Cox-2-expressing cells, in the presence of IPTG or without, proliferated to nearly the same levels as control, vector only transfected cells in response to EGF. The CaspACE™ Assay System, Fluorometric, was used to assay apoptosis after withdrawal of NGF.

For additional peer-reviewed articles that cite use of the CaspACE™ Assay System, Fluorometric, visit: [www.promega.com/citations](http://www.promega.com/citations)

## 2. Product Components and Storage Conditions

Product	Size	Cat.#
CaspACE™ Assay System, Fluorometric	160 assays	G3540

For Laboratory Use. Each system contains sufficient reagents to perform at least 160 assays (standard format) or 800 reactions (96-well plate format). Includes:

- 400 $\mu$ l ICE Substrate Ac-YVAD-AMC (10mM)
- 400 $\mu$ l CPP32 Substrate Ac-DEVD-AMC (10mM)
- 400 $\mu$ l ICE Inhibitor Ac-YVAD-CHO (10mM)
- 400 $\mu$ l CPP32 Inhibitor Ac-DEVD-CHO (10mM)
- 5mg AMC Standard (7-amino-4-methyl coumarin)
- 2  $\times$  30ml Caspase Assay Buffer

**Storage and Stability:** Store the CaspACE™ Assay System, Fluorometric, protected from light and moisture, at  $-20^{\circ}\text{C}$ . Avoid multiple freeze-thaw cycles. Store substrates and inhibitors in aliquots at  $-20^{\circ}\text{C}$ .

### 3. Reagent Preparation

Please read the following protocol thoroughly before using the CaspACET™ Assay System, Fluorometric. Directions are given to perform the assay in a total volume of 500µl using microcentrifuge tubes and a spectrofluorometer (standard assay format) or 100µl using 96-well plates and a fluorescence plate reader (96-well plate assay format). Be sure to note the differences in protocols and equipment necessary for each format.

#### Materials to Be Supplied by the User

- 30°C incubator (or 37°C incubator; see Notes, Sections 4.B and 4.C)
- spectrofluorometer
- 96-well plate and fluorescence plate reader (if using 96-well plate format)
- dimethyl sulfoxide (DMSO)
- DTT, 100mM
- deionized water
- Parafilm® laboratory film or plate sealer

**Substrates and Inhibitors:** Thaw the 10mM substrate and inhibitor stock solutions to room temperature. Dilute the desired amount of these solutions to 2.5mM with DMSO before use. Store the diluted stock solutions, protected from light and moisture, at -20°C. Avoid multiple freeze-thaw cycles.

**DTT:** Prepare a 1M DTT stock solution in deionized water. Dispense into aliquots, and store at -20°C. Dilute 1:10 with deionized water to make a 100mM solution before use.

### 4. Assay for CPP32/ICE Activity in Cell Extracts

#### 4.A. Assay Conditions

Use the following assay conditions to determine the specific activity of CPP32/ICE-like enzymes in cell extracts:

- **blank:** substrate only
- **assay:** substrate + enzyme source
- **negative control:** substrate + enzyme source + inhibitor

**Negative control** reactions are optional when determining the activity of purified ICE or CPP32 enzymes. Examples of preparation and analysis of cell extracts that can be used as **positive controls** are given in Section 5.

#### Notes:

1. Prior to starting the assay, thaw the Caspase Assay Buffer, substrates and inhibitors at room temperature and mix thoroughly.

- For optimal results, assaying several concentrations of the sample may be necessary. You may add up to 20 $\mu$ l of cell extract per reaction (150–500 $\mu$ g total protein) in the standard format and up to 10 $\mu$ l of cell extract per reaction (75–100 $\mu$ g total protein) in the 96-well format. If necessary, the sample may be diluted in Caspase Assay Buffer. Test each sample concentration under all three assay conditions. For example protocols for the preparation of cell extracts, see Sections 5.A and 5.B.
- The amount of cell extract used in assay and negative control reactions must be identical.

#### 4.B. Standard Assay (500 $\mu$ l reactions)

- Prepare duplicate microcentrifuge tubes for each of the three assay conditions as shown.

Component	Blank	Assay	Negative Control
Caspase Assay Buffer	160 $\mu$ l	160 $\mu$ l	160 $\mu$ l
DMSO	10 $\mu$ l	10 $\mu$ l	-
DTT, 100mM	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l
cell extract	-	X $\mu$ l	X $\mu$ l
2.5mM appropriate inhibitor	-	-	10 $\mu$ l
deionized water to final volume	490 $\mu$ l	490 $\mu$ l	490 $\mu$ l

- !** Use the appropriate inhibitor. CPP32/caspase-3 assays required the CPP32 Inhibitor (Ac-DEVD-CHO); ICE/caspase-1 assays require the ICE Inhibitor (Ac-YVAD-CHO).
- Mix the contents of the tubes by vortexing gently. Incubate at 30°C for 30 minutes.
  - Add 10 $\mu$ l of the appropriate 2.5mM substrate to each tube. For CPP32 (caspase-3) assays, use the CPP32 Substrate (Ac-DEVD-AMC); for ICE (caspase-1) assays, use the ICE Substrate (Ac-YVAD-AMC).
  - Incubate at 30°C for 60 minutes. Measure fluorescence of each reaction at an excitation wavelength of 360nm and emission wavelength of 460nm. Fluorescence measurements must be completed within 2 hours after adding substrate to stay within the linear range of the assay.

#### Notes:

- If desired, Steps 2 and 4 may be performed at 37°C.
- Many spectrofluorometers are limited in the number of wavelengths at which they can read. This assay is optimized using an excitation wavelength of 360nm and emission wavelength of 460nm; however, excitation wavelengths of 360–380nm and emission wavelengths of 460–480nm may be used to monitor these reactions (13).

#### 4.C. 96-Well Plate Assay (100µl reactions)

This protocol is useful to test multiple samples. The assay is performed directly in 96-well plates, and fluorescence of each sample is read using a fluorescence plate reader. Use a flat-bottom, white or black polystyrene 96-well plate for the assay.

Examples of preparation and analysis of cell extracts that can be used as **positive controls** are given in Section 5.

1. Prepare duplicate wells containing blank, assay and negative control reaction mixtures as follows:

Component	Blank	Assay	Negative Control
Caspase Assay Buffer	32µl	32µl	32µl
DMSO	2µl	2µl	-
DTT, 100mM	10µl	10µl	10µl
cell extract	-	Xµl	Xµl
2.5mM appropriate inhibitor	-	-	2µl
deionized water to final volume	98µl	98µl	98µl

- !** Use the appropriate inhibitor. CPP32/caspase-3 assays require the CPP32 Inhibitor (Ac-DEVD-CHO); ICE/caspase-1 assays require the ICE Inhibitor (Ac-YVAD-CHO).
2. Cover the plate with Parafilm® laboratory film or a plate sealer, and incubate at 30°C for 30 minutes.
  3. Add 2µl of the appropriate 2.5mM substrate to all wells. For CPP32 assays, use the CPP32 Substrate (Ac-DEVD-AMC); for ICE assays, use the ICE Substrate (Ac-YVAD-AMC).
  4. Cover the plate with Parafilm® laboratory film or a plate sealer, and incubate at 30°C for 60 minutes. Measure fluorescence of the reactions at an excitation wavelength of 360nm and emission wavelength of 460nm. Fluorescence measurements must be completed within 2 hours of substrate addition to stay within the linear range of the assay.

#### Notes:

1. If desired, Steps 2 and 4 may be performed at 37°C.
2. The 96-well plate assay is configured for an opaque plate that has a maximum well volume of approximately 300-500µl.
3. Plate readers generally perform best using plates with flat-bottomed wells.

## 5. Positive Controls

Apoptosis can be induced in experimental systems by a variety of methods that lead to caspase activation. These include:

- Treating Fas or TNF receptor-bearing cells with cross-linking or agonistic antibodies (14,15).
- Treating cells with DNA topoisomerase inhibitors, e.g., etoposide (16), with the protein kinase inhibitor staurosporine (17) or with microtubule-damaging agents such as paclitaxel (18).
- Exposing cells to genotoxic damage induced by ionizing radiation (18,19).

### 5.A. Example 1: Analysis of Caspase Activity in Jurkat Cells Treated with Anti-Fas Antibody

#### Materials to Be Supplied by the User

(Solution compositions are provided in Section 9.)


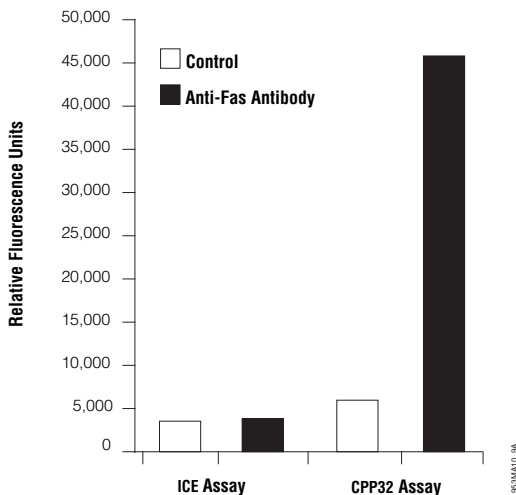
- hypotonic cell lysis buffer
  - anti-Fas monoclonal antibody (Clone# CH-11, MBL International Cat.# SY-001)
1. Grow Jurkat cells in RPMI 1640 medium containing 10% fetal bovine serum in a humidified, 5% CO<sub>2</sub> incubator at 37°C.
  2. Adjust the cell density to  $5 \times 10^5$  cells/ml. Add 100ng/ml of anti-Fas mAb (CH-11), and incubate for 4 hours at 37°C in a humidified, 5% CO<sub>2</sub> atmosphere.
-  As a control, concurrently incubate a culture treated with PBS only.
3. Harvest cells by centrifugation at  $450 \times g$  for 10 minutes at 4°C. Keep the cell pellet on ice.  
**Note:** Remove adherent cell lines from the plastic by trypsinization or scraping into ice-cold PBS.
  4. Wash 1X with ice-cold PBS, and resuspend in hypotonic cell lysis buffer at a concentration of  $10^8$  cells/ml.
  5. Lyse cells by subjecting them to four cycles of freezing and thawing. Centrifuge cell lysates at  $16,000 \times g$  for 20 minutes at 4°C, and collect the supernatant fraction.
  6. Measure CPP32 (caspase-3) and/or ICE (caspase-1) activity of anti-Fas antibody-treated (positive control) and untreated (control) cell lysates as described in Section 4.B (standard assay) or Section 4.C (96-well plate assay), Steps 1-4. Use extract from at least  $1 \times 10^6$  cells/assay.

Figure 1 shows a comparison of ICE (caspase-1) and CPP32 (caspase-3) activities in anti-Fas antibody-treated Jurkat T-cells.



**Figure 1. Measurement of ICE (caspase-1) and CPP32 (caspase-3) protease activities in anti-Fas antibody-treated human Jurkat T-cells.** Jurkat T-cells ( $5 \times 10^5$  cells/ml) were treated with 100ng/ml of anti-Fas mAb antibody or PBS (control) for 4 hours at 37°C. Cell lysates were tested for ICE and CPP32 activity according to the assay conditions described in Section 4.

### 5.B. Example 2: Analysis of Caspase Activity in THP-1 Cell Lysates

Cytosolic extracts prepared from the human monocytic leukemia cell line THP-1 (ATCC #TIB-202) may be used as a source of ICE and CPP32 enzymes (positive control).

1. Culture THP-1 cells in Iscove's Modified DMEM containing 9% horse serum, 2mM glutamine,  $2 \times 10^{-5}$ M  $\beta$ -mercaptoethanol and 1% penicillin-streptomycin in a 10% CO<sub>2</sub> incubator at 37°C. Grow cells to a density of  $5-6 \times 10^5$  cells/ml.
2. Harvest cells, and prepare cell extracts as described in Section 5.A, Steps 3-5.
3. Pre-activate the THP-1 cell extract by incubating at 37°C for 1 hour. Keep the pre-activated extract on ice until ready to use.
4. Measure the CPP32 and/or ICE activity of the pre-activated THP-1 cell lysate as described in Section 4.B (standard assay) or Section 4.C (96-well plate assay), Steps 1-4. Use extract from at least  $1 \times 10^6$  cells per assay.
5. Measure the protein content of the cell extract.

## 6. Calculation of Results

Calculate relative **fluorescence units** ( $\Delta$ FU) for each sample as described below. Because duplicate assays are done for each sample, determine the mean fluorescence values,  $\Delta$ FU1 (change in fluorescence in the absence of inhibitor) and  $\Delta$ FU2 (change in fluorescence in the presence of inhibitor) as follows:

$$\Delta\text{FU1} = (\text{mean assay FU}) - (\text{mean blank FU})$$

$$\Delta\text{FU2} = (\text{mean negative control FU}) - (\text{mean blank FU})$$

Calculate the value for  $(\Delta\text{FU1} - \Delta\text{FU2})/\text{time}$  to determine the change in fluorescence units per unit time at 30°C due to ICE/CP32 enzyme activity.

**Note:** If  $\Delta$ FU1 value is low, the assay may be allowed to proceed longer.

Calibrate a spectrofluorometer or fluorescence plate reader (for 96-well plate assays) using known amounts of AMC Standard solution as described in Section 7. Calculate the enzyme specific activity as described in Section 7.D.

## 7. AMC Calibration Curves

### 7.A. Preparation of AMC Stock Solutions

1. Prepare a 10mM stock solution of AMC by adding 2.85ml DMSO to the vial containing 5mg of AMC Standard supplied.

To confirm the AMC concentration, dilute the 10mM solution 1:400 in water and read the absorbance at 354nm. The concentration can be calculated using an extinction coefficient of  $16 \times 10^{-3}\text{M}^{-1}\text{cm}^{-1}$  as follows:

$$\frac{A_{354} \times 400}{16} = \text{concentration (mM)}$$

2. Dilute the AMC stock solution to 1mM. Perform serial dilutions of this 1mM stock solution in DMSO by combining the volumes shown in Table 1 to make 200 $\mu$ M, 100 $\mu$ M, 10 $\mu$ M, 2 $\mu$ M, 1 $\mu$ M and 0.2 $\mu$ M stock solutions.

**Table 1. Preparation of AMC Stock Solutions.**

AMC Stock Solution					Final AMC
1mM	100 $\mu$ M	10 $\mu$ M	1 $\mu$ M	DMSO	Solution
20 $\mu$ l	-	-	-	80 $\mu$ l	200 $\mu$ M
10 $\mu$ l	-	-	-	90 $\mu$ l	100 $\mu$ M
-	10 $\mu$ l	-	-	90 $\mu$ l	10 $\mu$ M
-	-	20 $\mu$ l	-	80 $\mu$ l	2 $\mu$ M
-	-	10 $\mu$ l	-	90 $\mu$ l	1 $\mu$ M
-	-	-	20 $\mu$ l	80 $\mu$ l	0.2 $\mu$ M

### 7.B. AMC Calibration Curve for Standard Assay (500µl reactions)

1. Using the AMC stock solutions described above, prepare 500µl of each AMC Standard (in duplicate) as described in Table 2.
2. Using a spectrofluorometer, measure the fluorescence of each sample using an excitation wavelength of 360nm and an emission wavelength of 460nm.
3. Plot fluorescence units (Y axis) versus AMC Standard in picomoles (X axis). The graph should be linear.
4. Calculate the slope of the calibration curve.

**Table 2. Components Required for AMC Calibration Curve (Standard Assay).**

AMC Standard	Picomoles AMC/500µl	AMC Stock Solution				DMSO	Caspase Assay Buffer	Deionized Water
		1µM	10µM	100µM	1mM			
0	0	-	-	-	-	10µl	160µl	330µl
10nM	5	5µl	-	-	-	5µl	160µl	330µl
100nM	50	-	5µl	-	-	5µl	160µl	330µl
200nM	100	-	10µl	-	-	-	160µl	330µl
500nM	250	-	-	2.5µl	-	7.5µl	160µl	330µl
1µM	500	-	-	5µl	-	5µl	160µl	330µl
10µM	5,000	-	-	-	5µl	5µl	160µl	330µl
20µM	10,000	-	-	-	10µl	-	160µl	330µl

### 7.C. AMC Calibration Curve for 96-Well Plate Assay (100µl reactions)

1. Prepare 100µl of each AMC Standard (in duplicate) as described in Table 3.
2. Using a fluorescence plate reader, measure the fluorescence of each sample using an excitation wavelength of 360nm and emission wavelength of 460nm.
3. Plot fluorescence units (Y axis) versus AMC Standard in picomoles (X axis). The graph should be linear.
4. Calculate the slope of the calibration curve.

**Table 3. Components Required for AMC Calibration Curve (96-well Plate Assay)**

AMC Standard	Picomoles AMC/100µl	AMC Stock Solution				DMSO	Caspase Assay Buffer	Deionized Water
		0.2µM	2µM	10µM	200µM			
0	0	-	-	-	-	10µl	32µl	58µl
10nM	1	5µl	-	-	-	5µl	32µl	58µl
100nM	10	-	5µl	-	-	5µl	32µl	58µl
200nM	20	-	10µl	-	-	-	32µl	58µl
500nM	50	-	-	5µl	-	5µl	32µl	58µl
1µM	100	-	-	10µl	-	-	32µl	58µl
10µM	1,000	-	-	-	5µl	5µl	32µl	58µl
20µM	2,000	-	-	-	10µl	-	32µl	58µl

### 7.D. Calculation of Enzyme Specific Activity

1. Estimate the **protein concentration** of each cell extract using BSA as a standard (20).
2. Use the following formula to calculate the activity of ICE-like enzymes present in a cell extract ( $\Delta$ FU1 and  $\Delta$ FU2 are defined in Section 6).

$X =$  pmol AMC liberated per minute in the **absence** of inhibitor

$$X = \frac{\Delta\text{FU1} - (\text{Y axis intercept})}{\text{time in minutes}} \times (\text{calculated curve slope})^{-1}$$

$Y =$  pmol AMC liberated per minute in the **presence** of inhibitor

$$Y = \frac{\Delta\text{FU2} - (\text{Y axis intercept})}{\text{time in minutes}} \times (\text{calculated standard curve slope})^{-1}$$

$$\text{Caspase activity} = \frac{(X-Y)}{\text{protein } (\mu\text{g})} \text{ pmol AMC liberated/minute at } 30^{\circ}\text{C}/\mu\text{g protein}$$

## 8. Additional Applications of ICE and CPP32 Inhibitors

Inhibitory constants ( $K_i$  values) for the ICE Inhibitor (Ac-YVAD-CHO) and CPP32 Inhibitor (Ac-DEVD-CHO) will vary depending on the particular ICE-like enzyme involved. For example, Ac-DEVD-CHO is a potent inhibitor of CPP32 ( $K_i < 1\text{nM}$ ) and a weak inhibitor of ICE ( $K_i = 10\mu\text{M}$ ). Similarly, Ac-YVAD-CHO is a potent inhibitor of ICE ( $K_i = 0.76\text{nM}$ ) and a significantly less effective inhibitor of CPP32 ( $K_i = 20\text{nM}$ ).

The difference in potency of these inhibitors may be used to discriminate between ICE and CPP32 enzyme activities present in cell lysates in either biological substrate (e.g., poly-[ADP-ribose] polymerase) cleavage assays with apoptotic cell lysates (2,14) or in DNA fragmentation analysis after incubation of rat thymocyte nuclei with apoptotic cell lysates (14,21,22). Assays are performed in the presence and absence of a range of concentrations (5nM–50 $\mu\text{M}$ ) of each inhibitor. The inhibitor that is capable of suppressing apoptosis at the lowest concentration is indicative of the enzyme present. For example, if the CPP32 Inhibitor can inhibit DNA fragmentation or PARP cleavage activity at a concentration of 5nM and the ICE Inhibitor is only effective at a concentration of 50 $\mu\text{M}$ , the enzyme present in the cell lysate is CPP32 (23).

**Note:** Concentrations of CPP32 Inhibitor or ICE Inhibitor in the range of 120–150 $\mu\text{M}$  are required to inhibit CPP32 or ICE enzyme activities in whole cell culture systems (14).

## 9. Composition of Buffers and Solutions

<b>Caspase Assay Buffer</b>	<b>hypotonic cell lysis buffer</b>
312.5mM HEPES (pH 7.5)	25mM HEPES (pH 7.5)
31.25% sucrose	5mM $\text{MgCl}_2$
0.3125% CHAPS (3-[(3-cholamido-propyl)-dimethyl ammonio]-1 propane sulfonate)	5mM EDTA
	5mM DTT
	2mM PMSF (phenyl methyl sulfonyl fluoride)
	10 $\mu\text{g}/\text{ml}$ Pepstatin A (Sigma Cat.# P4265)
	10 $\mu\text{g}/\text{ml}$ Leupeptin (Sigma Cat.# L2884)

## 10. References

1. Thornberry, N.A. *et al.* (1992) A novel heterodimeric cysteine protease is required for interleukin-1  $\beta$  processing in monocytes. *Nature* **356**, 768–74.
2. Nicholson, D.W. *et al.* (1995) Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* **376**, 37–43.
3. Tewari, M. *et al.* (1995) Yama/CPP32  $\beta$ , a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* **81**, 801–9.
4. Fernandes-Alnemri, T. *et al.* (1996) In vitro activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains. *Proc. Natl. Acad. Sci. USA* **93**, 7464–9.
5. Vaux, D.L. and Strasser, A. (1996) The molecular biology of apoptosis. *Proc. Natl. Acad. Sci. USA* **93**, 2239–44.
6. Kumar, S. and Lavin, M.F. (1996) The ICE family of cysteine proteases as effectors of cell death. *Cell Death Differ.* **3**, 255–67.
7. Nicholson, D.W. and Thornberry, N.A. (1997) Caspases: Killer proteases. *Trends Biochem. Sci.* **22**, 299–306.
8. Rosen, A. (1996) Huntingtin: New marker along the road to death? *Nat. Genet.* **13**, 380–2.
9. Yuan, J. *et al.* (1993) The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1  $\beta$ -converting enzyme. *Cell* **75**, 641–52.
10. Alnemri, E.S. *et al.* (1996) Human ICE/CED-3 protease nomenclature. *Cell* **87**, 171.
11. Humke, E.W., Ni, J. and Dixit, V.M. (1998) ERICE, a novel FLICE-activatable caspase. *J. Biol. Chem.* **273**, 15702–7.
12. Van de Craen, M. *et al.* (1998) Identification of a new caspase homologue: Caspase-14. *Cell Death Differ.* **5**, 838–46.
13. Thornberry, N.A. (1994) Interleukin-1  $\beta$  converting enzyme. *Meth. Enzymol.* **244**, 615–31.
14. Schlegel, J. *et al.* (1996) CPP32/apopain is a key interleukin 1  $\beta$  converting enzyme-like protease involved in Fas-mediated apoptosis. *J. Biol. Chem.* **271**, 1841–4.
15. Jänicke, R.U. *et al.* (1996) Specific cleavage of the retinoblastoma protein by an ICE-like protease in apoptosis. *EMBO J.* **15**, 6969–78.
16. MacFarlane, M. *et al.* (1997) Processing/activation of at least four interleukin-1 $\beta$  converting enzyme-like proteases occurs during the execution phase of apoptosis in human monocytic tumor cells. *J. Cell Biol.* **137**, 469–79.
17. Weil, M. *et al.* (1996) Constitutive expression of the machinery for programmed cell death. *J. Cell. Biol.* **133**, 1053–9.
18. Strobel, T. *et al.* (1997) Radiation-induced apoptosis is not enhanced by expression of either p53 or BAX in SW626 ovarian cancer cells. *Oncogene* **14**, 2753–8.
19. Datta, R. *et al.* (1997) Activation of a CrmA-insensitive, p35-sensitive pathway in ionizing radiation-induced apoptosis. *J. Biol. Chem.* **272**, 1965–9.

## 10. References (continued)

20. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-54.
21. Schlegel, J., Peters. I. and Orrenius S. (1995) Isolation and partial characterization of a protease involved in Fas-induced apoptosis. *FEBS Lett.* **364**, 139-42.
22. Lazebnik, Y.A. *et al.* (1994) Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature* **371**, 346-7.
23. Shimizu, S. *et al.* (1996) Bcl-2 expression prevents activation of the ICE protease cascade. *Oncogene* **12**, 2251-7.

## 11. Related Products

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay*	2.5ml <sup>1</sup>	G8090
Caspase-Glo® 8 Assay*	2.5ml <sup>1</sup>	G8200
Caspase-Glo® 9 Assay*	2.5ml <sup>1</sup>	G8210
Apo-ONE® Homogeneous Caspase-3/7 Assay	10ml <sup>1</sup>	G7790
CaspACET™ FITC-VAD-FMK In Situ Marker	50µl <sup>1</sup>	G7461
CaspACET™ Assay System, Colorimetric*	100 assays <sup>1</sup>	G7220
DeadEnd™ Colorimetric TUNEL System	40 reactions <sup>1</sup>	G7130
Anti-PARP p85 Fragment pAb	50µl	G7341
DeadEnd™ Fluorometric TUNEL System	60 reactions	G3250
DeadEnd™ Colorimetric TUNEL System	20 reactions <sup>1</sup>	G7360
CellTiter-Blue® Cell Viability Assay	20ml <sup>1</sup>	G8080
CellTiter-Glo® Luminescent Cell Viability Assay	10ml <sup>1</sup>	G7570
CellTiter 96® AQueous One Solution		
Cell Proliferation Assay*	200 assays <sup>1</sup>	G3582
CellTiter 96® AQueous Non-Radioactive		
Cell Proliferation Assay*	1,000 assays <sup>1</sup>	G5421
CellTiter 96® Non-Radioactive Cell Proliferation Assay*	1,000 assays <sup>1</sup>	G4000
CytoTox-ONE™ Homogeneous Membrane Integrity Assay	200-800 assays <sup>1</sup>	G7890
CytoTox 96® Non-Radioactive Cytotoxicity Assay*	1,000 assays	G1780
MultiTox-Fluor Multiplex Cytotoxicity Assay*	10ml <sup>1</sup>	G9200
CytoTox-Fluor™ Cytotoxicity Assay*	10ml <sup>1</sup>	G9260
rhTNF-α	10µg	G5241
Terminal Deoxynucleotidyl Transferase (TdT), Recombinant*	300u <sup>1</sup>	M1871

\*For Laboratory Use. <sup>1</sup>Additional Sizes Available.

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