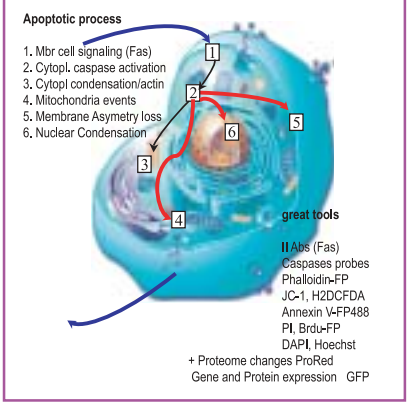
Apoptosis

Technical tip

Apoptosis is a programmed, cell-autonomous death process, known to impact every stage of life of multicellular eukaryotes. It is involved in a variety of physiological and pathological events, ranging from normal development (embryo growth, postnatal organ modeling and cell turnover) to diseases and disorders (cancer, organ failure and ageing, and neurodegenerative diseases). During apoptosis, the caspases execute the disassembly of cellular components by proteolytic cleavage of a variety of substrates, such as poly-(ADP ribose) polymerase (PARP), DNA-dependent protein kinase (DNA-PK), topoisomerases, and protein kinase C (PKC). At least ten caspases have been discovered. Some of these caspases identify and cleave a specific peptide substrate, while others recognize the same peptide substrate.

Selection guide



For your research Interchim offers a comprehensive line of products :

- Membrane assays (AnnexinV, AppolerCentage)
- Caspase assays
- Mitochondria assays

Method	Advantages	Disadvantages
Apoptosis membrane alteration : Dye (uptake bioassay: APOPercentage).	Single apoptotic cell (IHC). Quick and easy to use. Assay can also be quantified, using a digital camera or a microplate colorimeter/fluorimeter. Necrotic cells cannot retain the dye. Several hundred assays can be performed in one day.	Limited information available on cell membrane composition.
Apoptosis membrane alteration: (Annexin V binding)	Sensitivity Single cell (FCM, IHF). Confirms the occurrence of phosphatidylserine flippase in apoptotic cells, and the activity of initiator caspases.	Does not discriminate between apoptotic and necrotic cells.
Fluorescence Activated Cell Sorter (FACS).	Good option for suspension cells.	The equipment is designed for cell counting and cell sorting. Excessive physical force, to remove cell clumps, can damage cells. Lower Sensitivity (>100 apoptotic cells to discern cluster in a sample scan.
Apoptotic proteases (caspases assays)	It is possible to select for individual initiator caspases or execution caspases. Sensitivity : Single cell (with FCM) (IEA or IFA: >usually 1 x 10^5 cells) Detection and measurement of specific caspase activity.	Lysis or fixation of cells (except for CytoxiLux)Caspase activation does not necessarily imply that apoptosis will occur.
Enzymatic end labelling of DNA strand breaks (TdT TUNEL: TACS)	Can be completed within 3 hours. Fluorescence microscopy or FACS. Sensitivity. Single cell	Fixation of cells. Subject to false positives from necrotic cells and risk of high background from some viable cells. Collection times can be critical, early collection and DNA fragmentation may not yet be extensive, or later and DNA fragmentation can be excessive.
DNA fragmentation (BET/Electrophoresis : Commet Assay)	Photographic evidence of large DNA fragmentsSingle cell (with Comet Assay)	Can be difficult to produce a nucleosome ladder as further DNAfragmentation can occur during preparation. Necrotic cells also generate DNA fragments Sensitivity. 1 x 10^6 cells

Apoptosis Cell and Tissue Control Slides

Following control slides will help developing familiarity with the methods of in situ apoptosis labeling and to determine if reagents are working optimally.

Description	Cat.#	Qty
Cell Culture Control Slides	**Q69270**	2 u
	Q69271	5 u
Tissue Control Slides	**Q69290**	2 u
	Q69291	5 u

See also Antibodies Research Area #3)
(Apoptosis, and related Abs) and Last minute Abs (moitochondrial research).
See also M-PCR Kits

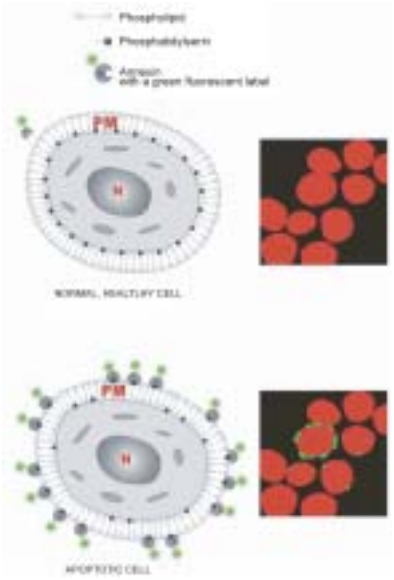
Membrane apoptosis events

Labeled Annexin V

Exposure of phosphatidyl serine

One of the first identifiable events during apoptosis is the «flipping» of phosphatidyl serine (PS) on the cell membrane, resulting in exposure of PS on the cell surface. The anticoagulant annexin V binds specifically to PS in a calcium-dependant event. PS translocation to the cell surface precedes nuclear breakdown, DNA fragmentation, and the appearance of most apoptosis-associated molecules, making annexin V binding a marker of apoptosis early-stage.
Annexin V is available conjugated with following labels by an optimized chemistry to ensure proper PS binfing.

Description	Cat.#	Qty
Annexin V - FluoProbes 488 for confocal microscopy 494/519 nm	**FP-BH4140**	500 µl
Annexin V - FluoProbes 488 for flow cytometry 494/519 nm	**FP-BH9390**	100 tests
Annexin V - FITC 494/518 nm	**FP-M19652**	250 µl
Annexin V - R-phycoerythrin (RPE) 496, 546, 565/578 nm	**FP-AH191A**	50 tests
Annexin V - Allo-phycocyanin (APC) 650/660 nm	**FP-AK194A**	50 tests
Annexin V - X - Biotin	**FP-M1969A**	500 µe
Related products : 30 fluorescents streptavidins can be chosen.	see page A350	
Anti - Annexin V - FITC 494/518 nm	**R51380**	100 tests
Annexin V, 10x binding buffer	**BU2080**	1.7 ml



Comparison of the performances of Annexin V- FluoProbes® 448 by flow cytometry and Annexin V-FITC.

The Jurkat cells are incubated with anti-Fas antibody for 3 hours to activate apoptosis. The activated cell suspension is then mixed with Annexin V- FluoProbes® 488 or Annexin V-FITC and further incubated for 15 minutes.

Figure 2 shows the fluorescence intensities histograms of the cells incubated with annexin V-FluoProbes® 488. Table lists the Mean Fluorescence Intensities of the viable cells (M1) and the apoptotic cells (M2) and the ratio of M2/M1 with annexin V- FluoProbes® 488 or annexin V-FITC.

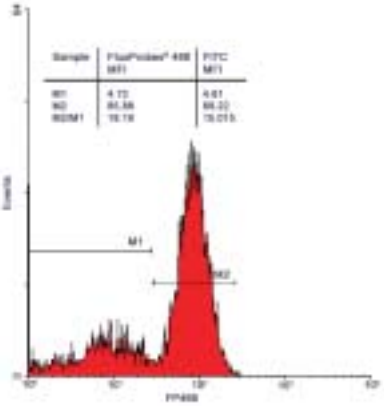
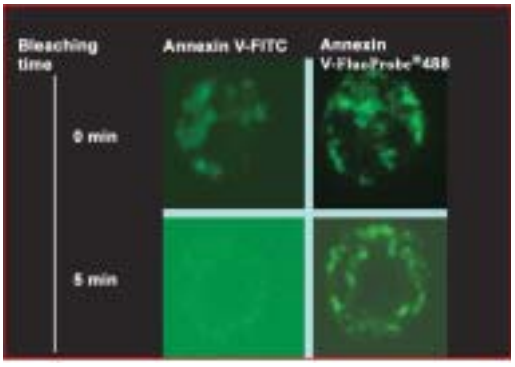
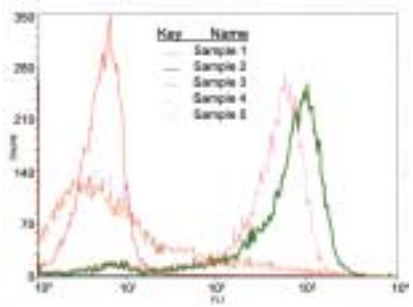


Figure 2 : Histogram of the cells incubated with annexin V-FP488

Associated product :
Prodium iodie Iodide FP-36774A 10 ml see page E123.
7.AAD FP-132303 1 mg see page E126 used for dead cell identification.



Performance of Annexin V-FITC and Annexin V-Fluoprobes 488 with Confocal Scanning Laser Microscopy.
The Annexin V-Fluoprobes 488 is superior to the FITC probe, both in quantum yield and in resistance to bleaching.



Flow Cytometry analysis of WEHI 7.1 cells labeled with Annexin V-Biotin and detected by streptavidin-FITC. WEHI 7.1 cells treated with 25 µm etoposide for two hours with an overnight recovery produce a peak approximately log of 10^3 in the fluorescence channel 1 (FL1) (samples 2 and 3). Healthy WEHI 7.1 cells produce a peak less than log 101 in the FL1 channel which is similar to unlabeled cells (samples 4.5 and 1 respectively). Analysis was tested using two different populations of cells (samples 1,2,4 and 3,5 respectively).

TACS™ Annexin V Kits

detection of apoptotic cells

Features :

- Fast, sensitive detection of apoptotic cells.
- Includes propidium iodide for discriminating apoptotic and necrotic (or late apoptotic) cells.
- Annexin V-Biotin provides flexibility in fluorophore choice.

TACS™ Annexin V Kits allow rapid, specific, and quantitative identification of apoptosis in individual cells. They use annexin V conjugated to either FITC or to biotin for the detection of cell surface changes during apoptosis. The annexin V conjugates are supplied with an optimized Binding Buffer and propidium iodide. Propidium iodide may be used on unfixed samples to determine the population of cells that have lost membrane integrity, an indication of late apoptosis or necrosis.

Description	Cat.#	Qty
TACS™ Annexin V Kits	**372810**	100 tests
	372811	250 tests

Related products :
Several complementatry probes are described in section E :

Description	Cat.#	Qty
PI see page E123	**FP-36774A**	10 ml @ 1 mg/ml
7-AAD is used for dead cell identification See page E126	**FP-132303**	1 mg

2 hr 4hr 6hr

Digital images of CHO cells treated with 10 mM cycloheximide, to induce apoptosis after 2, 4 and 6h.

APOPercentage™ APOPTOSIS Assay

The APOPercentage™ Assay is a detection and MEASUREMENT system to monitor the incidence of apoptosis in mammalian anchorage-dependent cells during *in vitro* culture.

Apoptotic cells take up the APOPercentage dye, as shown in the pictures below.

Following dye uptake, the level of apoptosis can be quantified colorimetrically, fluorometrically or by analytical digital photomicroscopy (ADP).

- Assay time : 1 hour
- Assay sensitivity : a single apoptotic cell.
- Compatible with CDD camera znd microplate readers

Description	Cat.#	Qty
APOPercentage™ Apoptosis Assay kits :		
Trial size Kit (3 x 96 wells; microwell format)	**R47770**	250 tests
Standard Assay Kit (6 x 96 wells; microwell format)	**R47771**	500 tests

Components of the # R47770 kit :
APOPercentage Dye reagent (5 ml) in phosphate buffered saline (sterile vial)
APOPercentage Dye Reference Standard (10 µM/10 ml), (sterile vial)
APOPercentage Dye Release reagent (150 ml)
Gelatin matrix forming solution (0.4%/100 ml / sterile)

Biochemicals for apoptosis research

Caspase inhibitor kit

Features :

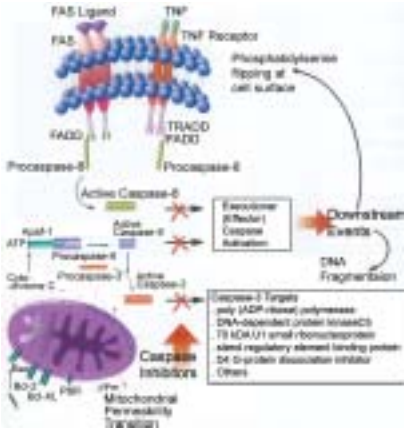
- Synthetic peptides coupled to fluoromethylketone (FMK) which irreversibly binds the caspase.
- High concentrations of inhibitor to reduce risks of DMSO toxicity.
- Each kit is provided with a negative control and a general caspase inhibitor.
- Application range from 0.2 to 200 µM.

Description	Cat.#	Qty
Caspase 3 Inhibitor Kit	**Q70200**	20 µl
Caspase 8 Inhibitor Kit	**Q70220**	20 µl
Caspase 9 Inhibitor Kit	**Q70240**	20 µl
Caspase 3,8,9 Inhibitor Kit	**Q70180**	20 µl Each

Apoptosis Inducers

Valinomycin is used to induce mitochondrial potential disruption. Etoposide is a DNA synthesis inhibitor that induces double stranded and single stranded DNA breaks. Staurosporine is a phospholipid/calcium-dependent protein kinase inhibitor that prevents ATP binding.

Description	Cat.#	Qty
Valinomycin	**FP-09246B**	25 mg
	092460	100 µl
Etoposide	**FX8650**	100 µl
Staurosporine	**74146D**	100 µg
	74146E	1 mg
	FX8660	100 µl



Schematic representation of the pathways of caspaces activation and where individual inhibitors will have an effect.

Technical tip

Applications :
Caspase inhibitors are useful tools, added to cell culture or cell-free extracts, for studying relationships between caspases and other factors involved in apoptosis. Caspase inhibitors inactivate specific caspases allowing study of their impact on pathways of interest, or of other caspases or apoptotic events. Among the events taking place during apoptosis are :

- Cytochrome c release into the cytoplasm,
- Caspase activation,
- Change of mitochondrial potential (Dym),

See also caspase inhibitors to add in cell cultures media.

Caspase Assays

Technical tip

Caspases 3 & 7

Caspase 3 and 7 are two closely related caspases with a central role in the exécution phase of apoptosis. They share common structure regions and substrate specificity (selectivity for the amino acid sequence Asp-Glu-Val-Asp (DEVD), but differ completely in the sequence of their respective N-terminal regions including their amino terminal peptides, 23-28 residue segments, which are cleaved during zymogen activation.

The activation of Caspase-3 (CPP32/apopain), is important for the initiation of apoptosis, and the enzyme is also identified as a drug-screening target.

Selection guide

Selection guide

Assay/Probe	Technique	Comment	page
TACS IB	FCM, IHF, FIA	FRET (FAM or SR/FMK) Inhibitory probes. Allows to quantitate caspase at end-point times	page E160
PhiPhiLux & probes for FCM / no CaspaLux	FCM, IHF	Superior fluorescnent cCell-permeabilization nor fixation	page E161
EnzoLyte Assays	FIA		page E162
Bioluminescent caspases probes		FIA	page E163

TACS™ Fluorescent Inhibitor Based (IB) Caspase Detection Assay

direct detection of apoptosis by analysing caspases activity

Features :

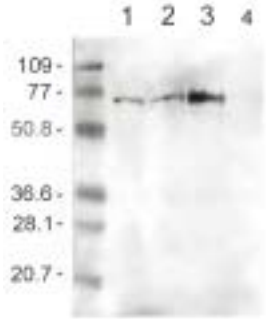
- Direct detection of apoptosis
- Simple procedure : incubate cells with reagent, wash, and detect

Each kit is based on established caspase inhibitor peptides. These labeled peptides are cell permeable and non-cytotoxic. The peptide documented sequences include 4 amino-acids (with a Asp residue) that are specifically recognized by caspase enzymes, and **irreversibly** binds the caspase. Cells can be analyzed by 96 well plate based fluorometry, fluorescence microscopy, or flow cytometry.

Caspase (Inhibitor Sequence)	Cat.#	Qty
Poly-Caspases (FAM-VAD-FMK)	**AJ298A**	25 tests
Poly-Caspases (FAM-VAD-FMK)	**AJ298B**	100 tests
Caspase 1 (FAM-YVAD-FMK)	**AJ299A**	25 tests
Caspase 1 (FAM-YVAD-FMK)	**AJ299B**	100 tests
Caspase 2 (FAM-VDVAD-FMK)	**AJ300A**	25 tests
Caspase 2 (FAM-VDVAD-FMK)	**AJ300B**	100 tests
Caspase 3 and 7 (FAM-DEVD-FMK)	**AJ301A**	25 tests
Caspase 3 and 7 (FAM-DEVD-FMK)	**AJ301B**	100 tests
Caspase 6 (FAM-VEID-FMK)	**AJ302A**	25 tests
Caspase 6 (FAM-VEID-FMK)	**AJ302B**	100 tests
Caspase 8 (FAM-LETD-FMK)	**AJ303A**	25 tests
Caspase 8 (FAM-LETD-FMK)	**AJ303B**	100 tests
Caspase 9 (FAM-LEHD-FMK)	**AJ304A**	25 tests
Caspase 9 (FAM-LEHD-FMK)	**AJ304B**	100 tests
Caspase 10 (FAM-AEVD-FMK)	**AJ305A**	25 tests
Caspase 10 (FAM-AEVD-FMK)	**AJ305B**	100 tests
Caspase 13 (FAM-LEED-FMK)	**AJ306A**	25 tests
Caspase 13 (FAM-LEED-FMK)	**AJ306B**	100 tests

SR (Red Fluorescence) Inhibitor Based (IB) Assays

Caspase (Inhibitor Sequence)	Cat.#	Qty
Poly-Caspases (SR-VAD-FMK)	**AJ307A**	25 tests
Poly-Caspases (SR-VAD-FMK)	**AJ307B**	100 tests
Caspase 3 and 7 (SR-DEVD-FMK)	**AJ308A**	25 tests
Caspase 3 and 7 (SR-DEVD-FMK)	**AJ308B**	100 tests
Caspase 9 (SR-LEHD-FMK)	**FX9070**	25 tests
Caspase 9 (SR-LEHD-FMK)	**FX9071**	100 tests



Induction of PPM1D/WIP1 in response to UV irradiation in human cells. Cryopreserved cells were plated 1 x 10^5 cells per ml. At 16 hours after plating, cells were exposed to 50J/m^2 of ultraviolet light (254 nm). At 2 hours after treatment, cells were harvested in PBS and analyzed by western blot using a 1 µg/mL dilution of anti-human PPM1D/WIP1 monoclonal antibody according to the protocol provided for colorimetric detection. Lane 1, UV treated Raji cells ; Lane 2, UV treated P3IB cell ; Lane 3, UV treated A549 cells ; and Lane 4, uninduced A549 cells.

PhiPhiLux® Caspases FCM Assays

PhiPhiLux® Caspase Assays are based on a novel and unique class of cell-permeable fluorogenic substrates, CaspaLux, for the detection and measurement of caspase 3 and caspase 3-like activities in living cells. The presence of the amino acid sequence of DEVDGI allows to determine the apoptosis specific, intracellular caspase-3 (CPP32) and caspase-3-like activities by flow cytometry or fluorescence microscopy. By judicious choice of fluorophores, either green or red fluorescence may be observed. This could also serve for caspase 7 study. Kits are available with either green or red fluorescence. Each kit of 30 tests (or 60 tests) contains 4 (or 8) vials of substrate and 1 (or 2) bottle of 60 ml flow cytometry dilution buffer, and complete protocols for FCM, standard Fluorescence microcopy, and Confocal microscopy applications.

Superior advantage over other assays and probes :

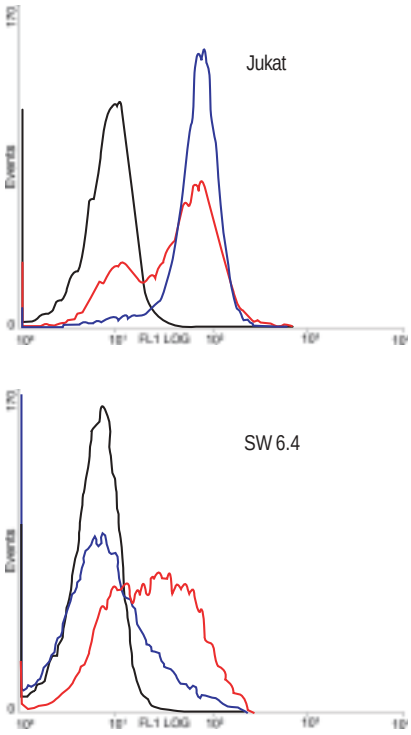
- Cells are neither permeabilized nor fixed
- Works not only for clear solutions but also cell suspensions

Description	Cat.#	Qty
PhiPhiLux®-3 - L_1D_2 Caspase 3 FCM assay	**U29491**	30 tests*
	U29492	60 tests(*)
Contains the L1D2 Caspalux-3 ($\lambda_{ex}/\lambda_{em}$: 505/530 nm		
PhiPhiLux®-3 – G_2D_2 Caspase 3 FCM assay	**BG4481**	30 tests*
	BG4482	60 tests(*)
Contains the G_2D_2 Caspalux-3 $\lambda_{ex}/\lambda_{em}$: 552/580 nm.		

Other caspase probes are available :

Other cell-permeable caspase probes are available, labeled with green ($\lambda_{ex}/\lambda_{em}$: 505/530 nm) and red fluorescence ($\lambda_{ex}/\lambda_{em}$: 542/580 nm):
CaspaLux®-6, is a cell-permeable fluorogenic substrate for Caspase 6, based on VEID sequence.
CaspaLux®-8, is a cell-permeable fluorogenic substrate for Caspase 8, based on IETDGI sequence.
CaspaLux®-9 is a cell-permeable fluorogenic substrate for Caspase 9, based on LEHDGI sequence.
Each item (30 tests) contains 4vials of labeled Caspalux, and 60 ml of FCM dilution buffer.
The CaspaLux®-1 - E_1D_2, cell-permeable substrate for Caspase 1 (BP7161), is available under licence.

Description	Cat.#	Qty
CaspaLux®-6 – J_1D_2: green cell-permeable substrate for Caspase 6	**BP7131**	30 tests
CaspaLux®-6 – J_2D_2: red cell-permeable substrate for Caspase 6	**BP7141**	30 tests
CaspaLux®-8 – L_1D_2: green cell-permeable substrate for Caspase 8	**BG4511**	30 tests
CaspaLux®-8 – L_2D_2: red cell-permeable substrate for Caspase 8	**BG4521**	30 tests
CaspaLux®-9 – M_1D_2: green cell-permeable green substrate for Caspase 9	**BG4531**	30 tests
CaspaLux®-9 – M_2D_2: red cell-permeable green substrate for Caspase 9	**BG4531**	30 tests

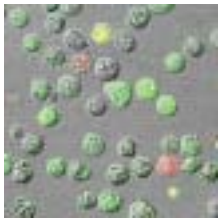


Sensitivity between a T-Cell leukemic (Jurkat cell) and a Burkitt's lymphoma (SKW 6.4) line to different apoptogens
Control, α Fas Ab, Staurosporine

Control

SW6.4 cell without (control) or with anti Fas Ab were imaged after probing with Phiphilux G1D2

With α Fas Ab



Apoptosis

Fluorescent Caspase Assays kits

Interchim provides microplate Caspases assays based on specific peptides substrates labeled by different fluorogenic labels.

Benefits include :

- Convenient Format : All essential assay components are included
- Enhanced Value : Less expensive than the sum of individual components
- High Speed : Minimal hands-on time
- Assured Reliability : Detailed protocol and references are provided
- Excellent sensitivity, specificity : fast and specific reaction with the provided substrate optimized conditions

The Caspase 3&7 kits use Ac-DEVD peptidic caspase substrate labeled with 4 following fluorogenic labels :

AFC and AMC are popular labels to monitor caspase activity. They yield blue fluorescence upon protease cleavage. Once cleaved they can be monitored at excitation/emission wavelengths = 354/442 nm (AMC) or 380/500 nm (AFC).

AnaRed™ yields near IR fluorescence upon proteolytic cleavage . $\lambda_{ex.}/\lambda_{em.}$: 618/625 nm

Magic Red™ emits near IR region upon proteolytic cleavage ($\lambda_{ex.}/\lambda_{em.}$: 585/610 nm). Its use has several advantages as compared to coumarin or rhodamine fluorophores including : (i) lower nonspecific autofluorescence signal associated with the longer wavelength excitation of Magic Red™, and (ii) fluorescent molecules excited at longer wavelengths are much less cytotoxic to the cell, thus preserving the cell viability status, which is important to live cell imaging studies.

Rh110 yields green fluorescence upon proteolytic cleavage ($\lambda_{ex.}/\lambda_{em.}$: 496/520 nm.). The longer-wavelength spectra and higher extinction coefficient of the green-fluorescent Rh110 label provides greater sensitivity and less interference from cell components.

The caspase 8 kit uses IETD peptidic substrates available labeled by Rh110 for fluorometric and colorimetric assays.

References
Thornberry N. A., et al., Science 281, 1312 (1998).
Reed J. C., J.Clin.Oncol. 17, 2941-2953 (1999).
Lazebnik Y. A., et al., Nature 371, 346 (1994).
Villa P., et al., Trends Biochem. Sci. 22, 388 (1997).

	AFC blue 380/500 nm	AMC blue 354/442 nm	AnaRed™ near IR 618/625 nm	Magic Red™* near IR 585/610 nm	Rh110 green 496/520 nm
Complete Kits					
Caspase Profiling Kit	**BE9840** (2 p)	**BF0020** (1 p)			
Caspase 3 Assay Kit (500 tests)	**BE9920**	**M1957A**	**HT1080**	**HT9580**	**85785A**£
Caspase 7 Assay Kit (500 tests)	**BE9970**	**BE9990**	**HS9070**	**HT1600**	**BF0010**
Caspase 8 Assay Kit (25 tests)					**BR4940**£
Kits without cell lysis buffer					
Caspase Substrate Sampler Kit	**BE9900** (8 x 100 tests)	**BF0050** (10 x 100 tests)			
Caspase 3 Screening Kit (500 tests)					**HT1330**

£ Also work as a colorimetric assay-also exist in other sizes and HTS format (#BR493)

* Magic Red™ is also known as cresyl violet (CV)

Each kit contains :
A 96-well plate coated with a series of AFC(2 plates) or AMC (1 plate) based caspase substrates along with various controls (positive and negative controls). A 384-well plate format is available on custom basis.

Cell lysis buffer
Assay buffer
AFC or AMC (fluorescence reference standard for calibration)

The EnzoLyte™ fluorogenic Caspase Profiling Kits contain 96-well plates pre-coated with a series of fluorogenic-based peptide substrates indicators for assaying caspase protease activities. They provide the best solution for profiling caspase or caspase inhibitors and are available with AFC #BE9840 and AMC labels (#BF0020).

EnzoLyte™ fluorogenic Caspase 3, 7, 8 Assay kits 5 EnzoLyte™ Caspase 3 and 7 Assay Kits contain the same DEVD substrate labeled by different fluorogenic labels (AFC, AMC, AmRed, MagicRed or Rh110) for assaying Caspase-3 activities and screening Caspase-3 inhibitors. They are optimized to detect Caspase-3 or 7 activities in cell culture directly without a time-consuming cell extraction step and purified enzyme preparations using a fluorescence microplate reader or fluorimeter. Each kit allow to perform 500 tests in standard microplates (up 1250 test can be assayed in a 384-well microplate).

Each kit contains :
Fluorogenic indicator
Cell lysis buffer
Assay buffer
Ac-DEVD-CHO (inhibitor)
Fluorescence reference standard for calibration

EnzoLyte™ Rh110 Caspase 3 Screening Kit "Most Sensitive"
The EnzoLyte™ Rh110 Caspase-3 Screening Kit contains (Cbz-DEVD)2Rh110 as a fluorogenic indicator for screening Caspase-3 inhibitors and inducers. The longer-wavelength spectra and higher extinction coefficient of the green-fluorescent Rh110 product provide greater sensitivity and less interference from cell components. The kit is used to continuously measure the activity of Caspase-3 using a fluorescence microplate reader. Cell lysis buffer is not provided

The kit contains as kit 85785A but without cell lysis buffer :
(Cbz-DEVD)2-Rh110 substrate (fluorogenic indicator)
Assay buffer
Ac-DEVD-CHO (inhibitor)
Rh110 (fluorescence reference standard for calibration)

The EnzoLyte™ fluorogenic Caspase Substrate Kit contains a series of AFC or AMC labeled peptide substrates as fluorogenic indicators for assaying caspase protease activities. It provides the best solution for identifying a suitable substrate for designing fluorogenic-based caspase assays. Cell lysis buffer is not provided.

Each kit contains :
10 AFC or AMC caspase substrates
Assay buffer
AMC (fluorescence reference standard for calibration) or
AFC (fluorescence reference standard for calibration)
A detailed protocol

Bioluminescent Caspase 3 Assays

Caspase-3 Luminescent Assay Kit provides a homogenous assay system for fast and ultra-sensitive detection of caspase-3 activity in purified enzyme or mammalian cell systems. This kit utilizes Ac-DEVD-amino-D-luciferin in combination with Firefly luciferase to detect caspase-3 activity. Upon substrate cleavage at the C-terminal side of the DEVD peptide sequence by caspase-3, amino-D-luciferin is released. Amino-D-luciferin serves as a substrate for Firefly luciferase enzyme, generating bioluminescence in the presence of oxygen, ATP and Mg2+. The intensity of emitted light is proportional to the caspase-3 activity.
Under optimal conditions, it is possible to detect <1 ng of a peptidase by using a bioluminogenic substrate.

Features :

- Ultra-sensitive : Detectable with fewer than 100 cells with excellent signal-to-noise ratio.
- Simple : Single-step homogenous assay.
- HTS-compatible : The extended-glow signal is compatible with HTS format

Description	Cat.#	Qty
Caspase-3 Bioluminescent Assay Kit	**BP8911**	2.5 mL
Caspase-3 Bioluminescent Assay Kit	**BP8912**	10 mL
Caspase-3 Bioluminescent Assay Kit	**BP8913**	100 mL

Contains :
Cell lysis/assay buffer
Enzyme substrate Ac-DEVD-amino-D-luciferin
Enzyme inhibitor Ac-DEVD-CHO

Mitochondria apoptosis events

Interchim provides several antibodies to several PTP proteins (see section A1), and immunocapture kits (see section J15 mitochondria probes). Please see our probes for mitochondrial study, including the famous JC1 membrane potential probe, which is available in following convenient kit.

JC-1 Mitochondrial membrane potential detection kit

Description	Cat.#	Qty
JC-1 mitochondrial membrane potential detection kit	**FP-52314B**	100 Tests

- 15 min to load cells with JC-1, rinse, and measure !
- Cell permeable and ratiometric dye (increased level and accuracy of signal)
- Direct measure of mitochondria membrane potential in living cells
- Application : apoptosis, mitochondria studies

JC-1 Mitochondrial Membrane Potential Detection Kit is used to measure mitochondrial membrane potential changes in cells, especially apoptosis Permeabilization Transition. In non-apoptotic cells, JC-1 accumulates as aggregates in the mitochondrial membranes (MPT), resulting in red fluorescence (590 nm). The brightness of red fluorescence is proportional to the potential and varies among different cell types. However, in apoptotic and necrotic cells, which have diminished mitochondrial membrane potential, JC-1 exists in the green fluorescent (529 nm) monomeric form [3-5]. This kit provides a step-by-step protocol and ready-to-use reagents (500 µL 100X JC-1 and
10 mL 10X buffer) for performing >100 assays for use on flow cytometers, fluorescence microscopes and fluorometric plate readers.

Related products
Probes include in above kits are available separately as well other useful probes :

Description	Cat.#	Qty	
JC1 $\lambda_{ex.}/\lambda_{em.}$: 514 / 529 nm	**FP-52314A**	5 mg	page E130
	FP-52314B	100 tests	
Rhodamine 123 $\lambda_{ex.}/\lambda_{em.}$: 505 / 534 nm	**FP-47372A**	50 mg	page E134
MitoRed stain $\lambda_{ex.}/\lambda_{em.}$: 560 / 580 nm	**FP-T32842**	8 x 50 µg	page E130
Staurosporine	**74176D**	100 µg	

Technical tip

Mitochondria are at the center of apoptosis, also known as programmed cell death. Intra-cellular and extra-cellular signals alter the association of a set of cytosolic pro-apoptotic and anti-apoptotic proteins with the organelle. These include the bax and bcl-2 families of proteins.

Alterations in the ratio of bax and bcl-2 like proteins regulate the permeability of the mitochondrial outer membrane and can result in the release of several proteins from the inter-membrane space, including cytochrome c, SMAC-Diablo and AIF (apoptosis inhibiting factor). Cytochrome c then reacts with a cytosolic protein APAF to induce an irreversible cascade of events involving caspases that lead to the orderly degradation of proteins and DNA.

A key contributor to the altered permeability of the organelle in apoptosis is the so-called **permeability transition** pore (Mito PT or PTP). This complex is thought to include hexokinase, porin and the peripheral benzodiazepine receptor of the outer membrane, adenylate kinase of the intermembrane space, ANT (the adenine nucleotide transporter) of the inner membrane and cyclophilin D from the matrix space.

See also MTT/XTT/UptiBlue Cell proliferation assays (to detect decreased Mitochondria activity during apoptosis)
See also Mitochondria proteins immunocapture kits in page E135.
See also AnnexinV FP488 page E157.

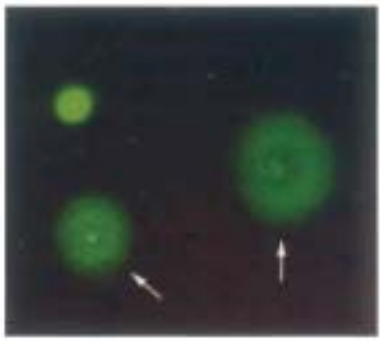
References :
1) Exp Hematol. 31, 815(2003); 2) Br J Haematol. 108, 574(2000); 3) Cytometry 29, 97(1997); 4) FEBS Lett 411, 77(1997); 5) J Neurochem 70, 66(1998).

Apoptosis

Technical tip

DNA fragmentation occurs as one of the final stages of cell death. DNA fragmentsare generated initially through single-stranded breaks then DNA fragments larger than 50 000 bases. Later in the process, dsDNA cleavage occurs mainly in the linker regions between nucleosomes, leaving ca 200 bases around the histone core, an a free 3' hydroxyl group. Simultaneously Poly(ADP-ribose) polymerase-1 (PARP-1) is activated by DNA breaks. Activated PARP-1 cleaves NAD into nicotinamide and ADP-ribose and catalyzes the transfer of ADP-ribose units from NAD+ to target nuclear proteins such as histones.
Methods for analyzing DNA fragments fall in following categories:
-DNA fragments evidenced by agarose electrophoresis, that more specially useful to evidence large DNA fragments. Limitation include DNA fragmentation can occur during preparation and in necrotic cells.
-DNA 3' termini evidenced by nucleotide incorporation via the deoxyNucleotidyl Transferase (TdT TUNEL). This rapid method may be subject to false positive from necrotic cells and risk of high background from some viable cells, and may show operating-dependnat reprocucibility.
-detection of the Poly(ADP-ribosyl)ation of proteins (PARP assays)

See the complete description of Comet Assay kit #815430 and other formats page E168



Results of a typical DASH™ Assay. Cells were treated with 100 mM H_2O_2 for 10 minutes at 4°C, subjected to the standard DASH™ protocol, and visualized using filters with a fluorescent microscope. The undamaged cell shows a compact, homogenous nucleoid, while the other two cells, indicated by arrows, show a nucleid with a typical diffuse halo pattern.

Interchim provides remarkable assays kits to remedy

Selection guide

Nuclear Apoptosis Event

Assay	Application	page
DNA fragmentation		
CometAssay	General : rapid detection and identification of DNA damage	page E168
DASH	General : discrimination of healthy and damaged (apoptotic or necrotic) cells	page D134
Nucleotide incorporation		
FlowTACS	General (Tdt TUNEL)	page E165
TiterTACS	Cell Culture	page E165
CardioTACS	Heart	page E166
NeuroTACS	Neurology	page E166
TumorTACS	Cancer	page E167
VasoTACS	Cardiology, Angiogenesis	page E168
DermaTACS	Cosmetology	page E167
TACS XL	General : Detect fragmented DNA (BrDu)	page E169
TACS™ Apoptotic DNA Laddering		page E169
PARP Assays		
Universal Colorimetric PARP Assay Kit	Measurement of PARP in cells and tissues Evaluation of PARP inhibitors	page E170

Nuclear apoptosis events – DNA fragmentation assays

CometAssay™ Single Cell Gel Electrophoresis Kit

Detect and quantitate DNA damage. Identify apoptotic cells quickly and easily.

DASH™ (Diffusion Apoptosis Slide Halo) Assay

discrimination of healthy and damaged (apoptotic or necrotic) cells

Advantages/Features :

- Identify apoptotic cells quickly and easily.
- Ready-to-use slides and reagents.
- Work with a small number of cells.

DASH™ (Diffusion Apoptosis Slide Halo) Assay kits allow discrimination of healthy and damaged (apoptotic or necrotic) cells in just a few hours. Simply embed cells in low-melting agarose on a pre-treated slide, lyse under alkaline conditions, and precipitate the DNA in the agarose. DNA from apoptotic cells diffuses away from the nucleoid and creates a characteristic halo pattern, while DNA from healthy, undamaged cells remains bright, compact, and homogeneous. The kit includes ready-to-use, pre-coated slides and optimized reagents for your convenience.

Description	Cat.#	Qty
DASH™ Kit	**FX8110**	50 Samples

Nuclear apoptosis events -nucleotide incorporation assays

FlowTACS

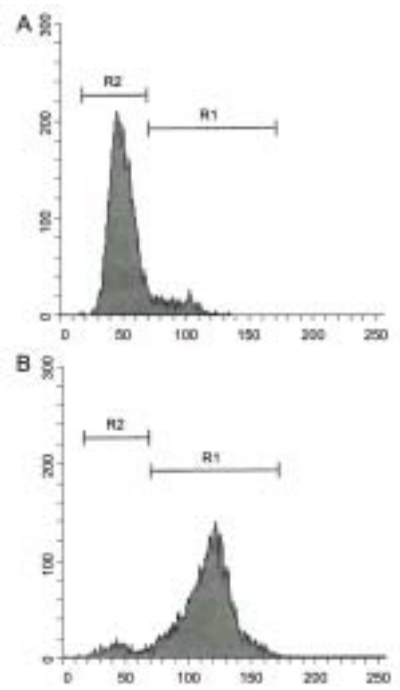
Identify and quantitate apoptotic cells in culture by bi-color and tri-color analysis.

Features :

- Fast. Requires less than 3 hours to complete.
- Exclusive, non-toxic TACS Safe TdT™ buffer - sodium cacodylate free.
- Unique buffer system produces more consistent labeling.
- Works on fixed cells.
- Includes exclusive Cytonin™ permeabilization reagent.
- Includes TACS-Nuclease™ solution for preparing sample-dependent positive controls.

FlowTACS™ provides flexibility in selection of fluorophores that are compatible with your research design. The kit allows multi-color labeling in conjunction with experiment-specific antibodies. DNA fragmentation is a committed step in apoptosis, and the labeling of 3' ends provides an easy measure of cells undergoing apoptosis. The FlowTACS™ Kit uses fixed cells, allowing you to safely work with cells that are infected with biohazardous agents. Also, samples may be stored conveniently during time-course experiments.

Description	Cat.#	Qty
FlowTACS™ Kit	**512510**	1 kit (60 Samples)
TACS™ 2 TdT DAB Kit	**Q69440**	30 Samples
TACS™ 2 TdT Blue Label Kit	**512520**	30 Samples
TACS™ 2 TdT Fluorescein Kit	**Q69470**	30 Samples
TACS™ 2 TdT Core Kit	**Q69430**	30 Samples
TACS™ 2 TdT Replenisher Kit	**Q69450**	30 Samples



Analysis of murine thymocytes at 16 hours after treatment with 10 µg/ml cycloheximide (A) and 1 µM dexamethasone (B). Cells were harvested and labeled according to the FlowTACS™ protocol prior to analysis by flow cytometry. Data courtesy N. Hardegen, NIH, NIDR, Bethesda, MD.

TiterTACS™ Colorimetric Apoptosis Detection Kit

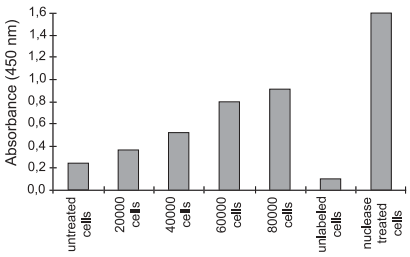
Identification and quantitation of apoptosis in cultured cells.

Features :

- Fast. Requires less than 4 hours to complete.
- Exclusive, non-toxic TACS Safe™ TdT buffer - sodium cacodylate free.
- Unique buffer system produces more consistent labeling.
- Includes exclusive Cytonin™ permeabilization reagent.
- Includes TACS-Nuclease solution for preparing sample-dependent positive controls.
- Convenient, 96 well microplate format.

The TiterTACS™ Colorimetric Apoptosis Detection Kit takes advantage of exclusive *in situ* labeling technology bringing it to the 96 well microplate format for high throughput quantitative detection of apoptosis. Detection using TACS-Sapphire™, a non-toxic colorimetric substrate, allows both kinetic and endpoint readings. The labeling of the 3' ends of DNA fragments provides an easy measure of cells undergoing apoptosis. Modified nucleotides are incorporated at the 3' ends by the activity of terminal deoxynucleotidyl transferase (TdT). These nucleotides are detected using a horseradish-peroxidase detection system and TACS-Sapphire™. TiterTACS™ can be used with suspension or monolayer cells. The kit is designed to use fixed samples, allowing you to work safely with samples that are infected with biohazardous agents. Fixed samples may be stored conveniently during time-course experiments.

Description	Cat.#	Qty
TiterTACS™ Colorimetric	**Q69590**	96 Samples



Detection of apoptosis in ML-1 cells after treatment with 1 µM staurosporine. All control wells contained 1 x 10^5 cells. Cells were harvested, fixed and labeled according to the TiterTACST™ protocol prior to colorimetric analysis. Reaction was stopped with 2N HCl.

NeuroTACS™ II kit

identification of apoptosis in brain tissue or neuronal cells

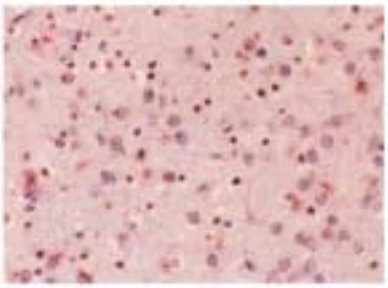
Features :

- Fast. Requires less than 3 hours to complete.
- Exclusive, non-toxic TACS Safe™ TdT buffer - sodium cacodylate free.
- Unique buffer system produces more consistent labeling.
- Performance tested on brain sections.
- Includes exclusive NeuroPore™ permeabilization reagent.
- Includes TACS-Nuclease™ solution for preparing sample-dependent positive controls.

NeuroTACS™ II is a complete reagent kit optimized to provide rapid and convenient identification of apoptosis in brain tissue or neuronal cells. The kit has been developed to overcome the common difficulties unique to neuronal samples including the fragile nature of brain tissue sections, high background problems, poor counterstaining with common dyes, and the need to perform dual labeling experiments to detect cell specific antigens in conjunction with apoptotic cells. A key feature is NeuroPore™, a proprietary permeabilization reagent that gently permeabilizes samples while retaining cell morphology. NeuroPore™ also contains blocking reagents to allow its use as an antibody diluent in immunohistochemistry and to reduce background staining. The protocol includes details for labeling *in situ* for apoptosis and antigen detection on the same sample.

Applications :

- *In situ* detection of apoptosis in fixed frozen, paraffin embedded, or plastic embedded cells and tissues.
- Assists in the identification of apoptotic morphologies.
- Helps resolve unique problems encountered when detecting apoptotic neuronal cells.



Double labeling of mouse brain section for apopotosis using NeuroTACS™ II (brown) and a monoclonal antibody to GFAP (red). Brain sections were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectionned at 5 µM. The section was counterstained using Trevigen's Blue Counterstain.

Description	Cat.#	Qty
NeuroTACS™ II Kit	**Q69600**	30 Samples

CardioTACS™ Kit

Identifying apoptotic cells in cardiac samples

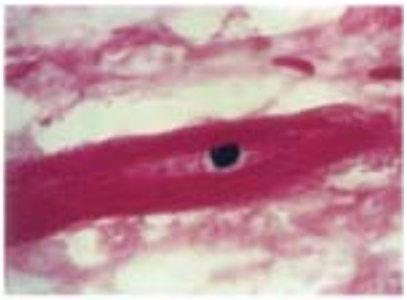
Features :

- Fast. Requires less than 3 hours to complete.
- Exclusive, non-toxic TACS Safe™ TdT buffer - sodium cacodylate free.
- Unique buffer system produces more consistent labeling.
- Performance tested on heart-derived samples.
- Includes exclusive Cytonin™ permeabilization reagent.
- Includes TACS-Nuclease™ solution for preparing sample-dependent positive controls.

The CardioTACS™ Kit was developed to provide the heart researcher with an effective method for identifying apoptotic cells in cardiac samples. The high cellularity of cardiac tissue presents problems in permeabilization, so the CardioTACS™ Kit comes with two permeabilization reagents to provide options. The kit is based on DNA end-labeling using terminal deoxynucleotidyl transferase (TdT) and a modified nucleotide that is subsequently detected using our TACS Blue Label™ detection system.

Applications :

- In situ detection of apoptosis in fixed frozen, paraffin embedded, or plastic embedded cardiac cells and tissues.
- Assists in the identification of apoptotic morphologies.
- Helps resolve unique problems encountered when detecting apoptotic cardiac cells.



Apoptotic rat cardiac myocyte labeled using the CardioTACS™ kit. Rat heart tissue was fixed in 4% paraformaldehyde avernight followed by paraffin embedding. Five micron sections were prepared and placed onto glass microscope slides. The sample was processed following the CardioTACS™ Kit protocol. photo courtesy Dr. J.Zhang, FDA.

Description	Cat.#	Qty
CardioTACS™ Kit	**820540**	30 Samples

DermaTACS™ Kit

an effective method for measuring apoptosis in skin samples.

Features :

- Fast. Requires less than 3 hours to complete.
- Exclusive, non-toxic TACS Safe™ TdT buffer - sodium cacodylate free.
- Unique buffer system produces more consistent labeling.
- Performance tested on skin-derived samples.
- Includes exclusive Cytonin™ permeabilization reagent.
- Includes TACS-Nuclease™ solution for preparing sample-dependent positive controls.

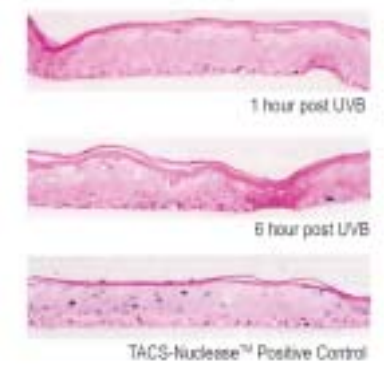
The DermaTACS™ Kit was developed to provide researchers with an effective method for measuring apoptosis in skin samples. The kit was based on DNA end-labeling using terminal deoxynucleotidyl transferase (TdT) and modified nucleotides. Detection of incorporated molecules is achieved using a chromogenic substrate with a horseradish peroxidase detection system. This complete kit provides all the reagents required for labeling including two permeabilization reagents, labeling and stop buffers, labeling and detection reagents, and TACSNuclease™.

Applications :

- In situ detection of apoptosis in fixed frozen, paraffin embedded, or plastic embedded cells and tissues.
- Assists in the identification of apoptotic morphologies.
- Helps resolve unique problems encountered when detecting apoptosis in skin sections.

Description	Cat.#	Qty
DermaTACS™ Kit	**Q69710**	30 Samples
EpiDerm™ Control Slides	**Q69310**	2 u

sections of synthetic human skin tailored for use with DermaTACS™



Detection of DNA fragmentation in UVB irradiated human skin model, EpiDerm™, with the in situ apoptosis detection lit for skin cells and tissues, DermaTACS™. The dark blue stained cells at 1 and 6 hours also exhibit punctate morphology indicative of apoptosis. The TACS-Nuclease™ treated sample shows the diffuse blue staining of fragmented but uncondensed DNA. Samples were provides courtesy of Dr. Patrick Hayden, MatTek Corporation, Ashland, MA.

TumorTACS™

Identification of apoptosis in tumors or cancer cells

Features :

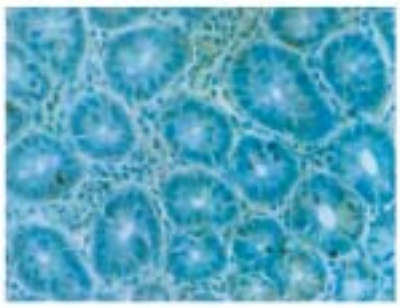
- Fast. Requires less than 3 hours to complete.
- Exclusive, non-toxic TACS Safe™ TdT buffer - sodium cacodylate free.
- Unique buffer system produces more consistent labeling.
- Performance tested on tumor samples.
- Includes exclusive Cytonin™ permeabilization reagent.
- Includes TACS-Nuclease™ solution for preparing sample-dependent positive controls.

TumorTACS™ is a complete reagent kit providing rapid and convenient identification of apoptosis in tumors or cancer cells. This kit has a unique TUNEL-based system that preferentially labels the double-stranded DNA found in apoptotic cells. DNA fragments generated during apoptosis are end-labeled with modified nucleotides using a highly purified terminal deoxynucleotidyl transferase enzyme (TdT) in a unique non-toxic labeling buffer supplemented with cations for apoptosis-specific labeling. The incorporated nucleotides are subsequently detected using a horseradish peroxidase conjugate. The conjugate catalyzes the conversion of diaminobenzidine (DAB) into a visible dark brown precipitate.

Applications :

- In situ detection of apoptosis in fixed frozen, paraffin embedded, or plastic embedded cells and tissues.
- Assists in the identification of apoptotic morphologies.
- Helps resolve unique problems encountered when using tissues or cells from tumors.

Description	Cat.#	Qty
TumorTACS™ Kit	**Q69480**	30 Samples



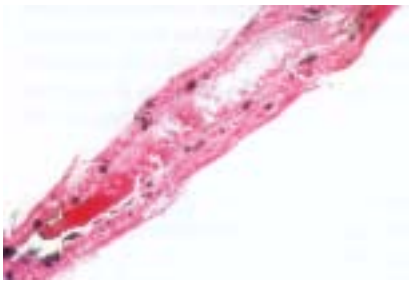
Apoptotic cells within mouse mammary tumor identified using the TumorTACS™ kit. Mammary tumor was fixed in 4% paraformaldehyde overnight followed by paraffin embedding. Five microns sections were prepared and placed onto glass microscope slides. The sample was processed following the TumorTACS™ kit protocol.

VasoTACS™ Kit

an effective method for identifying apoptotic cells in vascular samples

Features :

- Fast. Requires less than 3 hours to complete.
- Exclusive, non-toxic TACS Safe™ TdT buffer - sodium cacodylate free.
- Unique buffer system produces more consistent labeling.
- Performance tested on vascular tissues.
- Includes exclusive Cytonin™ permeabilization reagent.



Drug-induced apoptosis in the small artery of a rat exhibiting spontaneous hypertension using the VasoTACS™ Kit. The tissue was formalin-fixed and paraffin-embedded. Photo courtesy of Dr. Jun Zhang, FDA.

The VasoTACS™ Kit was developed to provide an effective method for identifying apoptotic cells in vascular samples. This kit allows the user to successfully label apoptotic cells, including endothelial and smooth muscle cells, throughout the vascular system. Similar to the CardioTACS™ Kit, this kit is also based on DNA end-labeling using terminal deoxynucleotidyl transferase (TdT) and a modified nucleotide that is subsequently detected using our TACS Blue Label™ detection system. Specifically, VasoTACS™ has been tested and optimized in order to help eliminate background and improve labeling in vascular tissue.

Applications :

- In situ detection of apoptosis in fixed frozen, paraffin embedded, or plastic embedded vascular cells and tissues.
- Assists in the identification of apoptotic morphologies.
- Helps resolve unique problems encountered when detecting apoptotic vascular cells.

Description	Cat.#	Qty
VasoTACS™ Kit	**T07640**	30 Samples

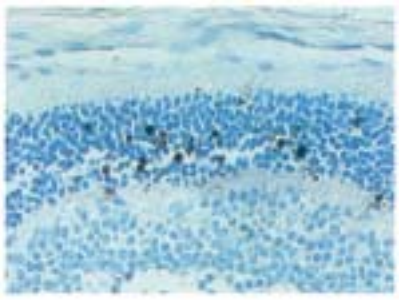
TACS-XL® In Situ Apoptosis Detection Kits

Detect fragmented DNA by analysing of incorporation of bromodeoxyuridine (BrdU).

Features :

- High signal-to-noise ratio generates stronger signal with less background.
- Less sensitive to protease-induced false positive labeling than digoxigenin or biotin-based kits.
- Complete kit provides either DAB or TACS Blue Label™ detection options.
- Includes exclusive Cytonin™ permeabilization reagent.
- Includes TACS-Nuclease™ control reagents.
- Readily adapted for fluorescence read-out.

TACS-XL® embodies a new approach for the *in situ* detection of apoptosis. The TACS-XL® kit is based on incorporation of bromodeoxyuridine (BrdU) at the 3' OH ends of the DNA fragments that are formed during apoptosis. The incorporation of BrdUTP by TdT is more efficient than either biotinylated or digoxigenin labeled nucleotides used in other TUNEL-based assays. The detection system utilizes a biotin conjugated anti-BrdU antibody and streptavidin-horseradish peroxidase. The combination of antibody specificity with the signal enhancing properties of biotin : streptavidin results in precise cellular labeling and the highest signal-to-noise ratio observed in competitive testing.



Detection of apoptosis cell of the photoreceptor layer of retina in 10-day-old FVB mice using the TACS-XL® DAB Kit. The tissue was fixed in 10% formalin and 5 µM paraffin sections were used for the assay (400x magnification). Photo courtesy Dr. S. Alikunju, Department of Cell Biology, Baylor College of Medicine, Houston, TX.

Applications :

- *In situ* detection of apoptosis in fixed frozen or paraffin embedded.
- Assists in the identification of apoptotic morphologies.

Description	Cat.#	Qty
TACS-XL® In SituApoptosis Detection Kits		
TACS-XL® Basic Kit	**Q69680**	30 Samples
TACS-XL® Blue Label Kit	**859790**	30 Samples
TACS-XL® DAB Kit	**Q69670**	30 Samples
TACS-XL® Replenisher Kit	**Q69700**	30 Samples
Blue Label Detection Module		
DAB Detection Module	**Q69660**	1 unit
Nuclease Module	**Q69690**	1 unit

TACS™ Apoptotic DNA Laddering Kits

The TACS™ Apoptotic DNA Laddering Kits are used to detect and estimate the level of internucleosomal DNA fragmentation that occurs during apoptosis. Kit selection is dependent upon the degree of apoptosis, number of cells, and availability of equipment. The evidence of DNA laddering supports other experimental data derived from morphological identification methods. Each kit contains all reagents necessary to isolate, label, and detect DNA. For those researchers investigating apoptosis in tissues, a supplemental Tissue Extraction Kit is available. This kit provides the reagents necessary to prepare tissues for DNA extraction.

Description	Cat.#	Qty
Ethidium Bromide DNA Laddering kit	**Q69850**	20 Samples
Isotopic DNA Laddering Kit	**Q69870**	20 Samples
Chemiluminescent DNA Laddering kit	**Q69930**	20 Samples
Colorimetric DNA Laddering Kit	**Q69950**	20 Samples
Tissue Extraction Reagents	**Q69960**	20 Samples

Nuclear apoptosis events -PARP assays

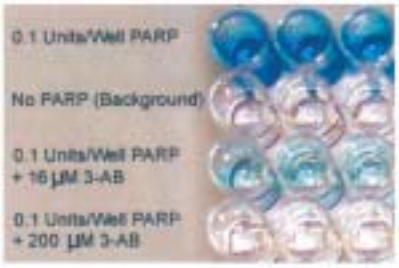
Activated PARP-1 cleaves NAD into nicotinamide and ADP-ribose and catalyzes the transfer of ADP-ribose units from NAD+ to target nuclear proteins. Poly(ADP-ribosyl)ation of proteins has been implicated in the regulation of a diverse array of cellular processes ranging from DNA repair and genetic stability to chromatin organization , transcription, replication and protein degradation) . Moderate activation of PARP-1 facilitates the efficient repair of DNA damage. However, overactivation of PARP has been implicated in the pathogenesis of several diseases, including stroke, myocardial infarction , diabetes , shock, neurodegenerative disorders and allergy PARP inhibitors show promise in improving cardiac and vascular dysfunction associated with advanced aging, preventing allergen-induced asthma-like reactions in sensitized Guinea pigs and in augmenting the activity of Topoisomerase inhibitors in the treatment of cancer.

Universal Colorimetric PARP Assay Kit

Measurement of PARP activity in cells and tissues. Evaluation of PARP inhibitors

Features :

- Colorimetric, non-radioactive format
- Higher throughput 96 test size in a 96 well microplate format
- Sensitivity down to 0.01 units of PARP per well



Poly ADP-ribosylation of nuclear proteins is a post-translational event that occurs in response to DNA damage. Poly (ADP-ribose) polymerase (PARP) catalyzes the NAD-dependent addition of ribose to adjacent nuclear proteins. Univeral Colorimetric PARP Assay Kit quickly measures the incorporation of a unique biotinylated NAD substrate onto histone proteins in a 96 well plate format. This technology is ideal for the screening of inhibitors of PARP where the formation of biotinylated poly (ADP-ribose) chains is inhibited and for measuring the activity of PARP in cell and tissue extracts. For your convenience, we offer two forms of the Universal 96 Well PARP Assay Kit :

1. Universal Colorimetric PARP Assay Kit with Histone Reagent. This format allows you to coat your own 96 well plate for maximum flexibility.

2. Universal Colorimetric PARP Assay Kit with Histone-Coated Plate. This format significantly reduces assay time and optimizes efficiency of your laboratory and personnel.

Description	Cat.#	Qty
Universal Colorimetric PARP Assay Kit with Histone reagent	**FX8230**	1 Kit
Universal Colorimetric PARP Assay Kit, with Histone-Coated 96 Well Plate (#FX8260)	**FX8250**	1 Kit
Histone-Coated 96 Well Plate	**FX8260**	1 Plate

Related product : FITC-NAD

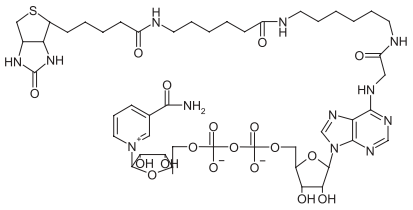
Applications :

- Activity measurements of NAD-requiring enzymes.
- Assays to identify inhibitors of activators of NAD-requiring enzymes.

FITC-NAD (6-Fluorescein-17-nicotinamide-adenine-dinucleotide) and Biotinylated NAD (6-biotin-17-nicotinamide-adenine-dinucleotide) provide a convenient non-isotopic alternative to radiolabeled NAD for use with enzymes requiring NAD as substrate or cofactor. A number of proteins, including poly (ADP-ribose) polymerase (PARP), and the SIR2 family of NAD(+)-dependent histone/protein deacetylases, use NAD as a substrate for their function. FITC-conjugatated NAD (provided with a proprietary permeabilization agent to favors cell loading) permits the direct measurement of PARP and other NAD-dependent enzymes by fluorescence microscopy or by incorporation of fluorescein-labeled poly (ADP-ribose) or O-acetyl-ADP ribose onto histones attached to 96 well plates. Biotinylated NAD allows an indirect measure of PARP activity when biotin incorporation is detected using a conjugated-streptavidin detection system.

Description	Cat.#	Qty
FITC-NAD	**FX8240**	250 µl
FITC-NAD	**FX8241**	5 x 250 µl
FITC-NAD	**FX8242**	10 x 250 µl

Description	Cat.#	Qty
Biotinylated NAD	**P56560**	500 µl
Biotinylated NAD	**P56561**	5 x 500 µl
Biotinylated NAD	**P56562**	10 x 500 µl



Related products : PARP Inhibitors

♦ 3-Aminobenzamide

3-aminobenzamide is the PARP inhibitor provided with Universal Colorimetric PARP Assay Kit at 200 mM.

♦ 4-Amino-1,8-naphthalimide

4-amino-1,8-naphthalimide is a potent inhibitor of poly (ADP-ribose) polymer (PARP) and reduces ischemia-reperfusion injury in the heart and skeletal muscle. It is 1000-fold more potent that 3-aminobenzamide and exhibits mixed-type inhibition with respect to the substrate, NAD+, at micromolar concentrations. This inhibitor, which has been analyzed at a concentration of 20 µM, is provided at a convenient concentration for subsequent serial dilution and use with Universal Colorimetric PARP Assay Kit.

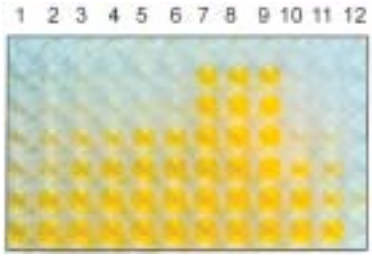
♦ 6(5H)-Phenanthridinone

6(5H)-Phenanthridinone strongly inhibits poly (ADP-ribose) polymerase (PARP) and displays immunosuppressive activity. It is a mixed-type inhibitor that acts on both the enzyme and enzyme-NAD+ complex at site(s) distinct from the NAD+-binding site to attenuate the fall in NAD and ATP and, consequently, improve cell survival. It also inhibits concanavalin A induced lymphocyte proliferation at micromolar concentrations. This inhibitor, which has been analyzed in the range of 0.18-0.39 µM, is provided at a convenient concentration for subsequent serial dilution and use with Universal Colorimetric PARP Assay Kit.

♦ Benzamide

Benzamide is the most potent poly (ADP-ribose) polymerase (PARP) inhibitor in the family of benzamides. It acts as a neuroprotectant since it inhibits PARP, an enzyme activated by nitric oxide. Benzamide is twice as active than its commonly used counterpart, 3-aminobenzamide in delaying or suppressing PARP activation. It is able to prevent nuclear fragmentation and apoptotic-body formation without affecting DNA fragmentation during apoptosis. This PARP inhibitor, which has been analyzed in the range of 100 to 500 µM, is provided at a convenient concentration for subsequent serial dilution and use with Universal Colorimetric PARP Assay Kit.

Description	Cat.#	Qty
3-Aminobenzamide	**Q69090**	60 µl
4-Amino-1,8-naphthalimide	**Q69120**	100 µl
6(5H)-Phenanthridinone	**Q69130**	100 µl
Benzamide	**Q69140**	100 µl



The presence of biotinylated NAD incorporated by PARP during the ribosylation of histone proteins layered on the surface of a microwell plate is detected using a streptavidin-horseradish peroxidase system and the TACS Sapphire™ substrate. Inhibitors 4-amino-1,8-naphthalimide (lane 1-3), benzamide (lane 4-6), 6(5H)-phenanthridinone (lane 7-9), and 3-aminobenzamide (lane 10-12) were used in decreasing concentration of inhibitor starting with the highest concentration at the top and no inhibitor used at the bottom.