#12145 Store at 4°C

Apoptosis and Proliferation Alexa Fluor® 488 Conjugated Antibody Sampler Kit

☑ 1 Kit
(4 x 40 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Isotype	Flow Cytometry Dilution	IF-IC Dilution
Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (Alexa Fluor® 488 Conjugate)	9603	40 µl	Rabbit IgG	1:50	1:50
Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate)	9148	40 µl	Rabbit IgG	1:50	1:50
Ki-67 (D3B5) Rabbit mAb (Alexa Fluor® 488 Conjugate)	11882	40 µl	Rabbit IgG	1:50	1:200
Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 488 Conjugate)	2975	40 µl	Rabbit IgG	N/A	N/A

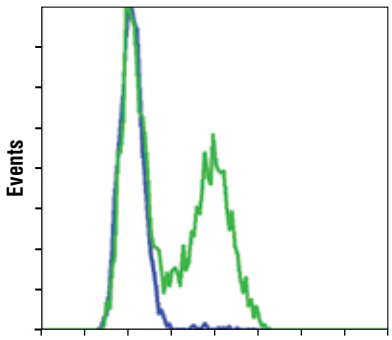
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Apoptosis and Proliferation Alexa Fluor® 488 Conjugated Antibody Sampler Kit provides an economical means to study apoptosis and proliferation status using known markers without the need for a fluorescent secondary antibody.

Background: Caspase-3 (CPP-32, Apoptain, Yama, SCA-1) is a critical executioner of apoptosis, as it is either partially or totally responsible for the proteolytic cleavage of many key proteins, such as the nuclear enzyme poly (ADP-ribose) polymerase (PARP) (1). PARP is involved in DNA repair in response to environmental stress (2). Ki-67 is a nuclear nonhistone protein that is universally expressed among proliferating cells and absent in quiescent cells (3,4). Ki-67 detects proliferating cells in G1, S, G2, and mitosis, but not in the G0 resting phase.

Specificity/Sensitivity: Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (Alexa Fluor® 488 Conjugate) recognizes endogenous levels of caspase-3 protein only when cleaved at Asp175. Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) recognizes endogenous levels of the large fragment (89 kDa) of human PARP1 protein produced by caspase cleavage. This antibody does not recognize full length PARP1 or other PARP isoforms. Ki-67 (D3B5) Rabbit mAb (Alexa Fluor® 488 Conjugate) recognizes endogenous levels of total Ki-67 protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp175 of human caspase-3 protein, a synthetic peptide corresponding to residues surrounding Asp214 of human PARP protein, or a recombinant protein specific to the amino terminus of Ki-67 protein.



Flow cytometric analysis of Jurkat cells, untreated (blue) or etoposide-treated (green), using ***Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #9148****.*

Background References:

(1) Fernandes-Alnemri, T. et al. (1994) *J Biol Chem* 269, 30761-4.

(2) Satoh, M.S. and Lindahl, T. (1992) *Nature* 356, 356-8.

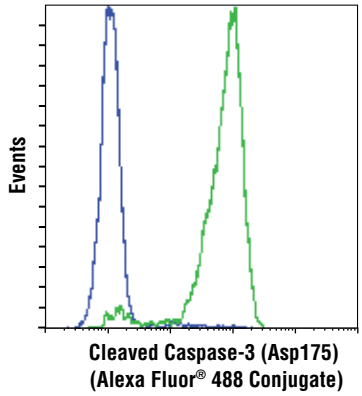
(3) Gerdes, J. et al. (1983) *Int J Cancer* 31, 13-20.

(4) Weigel, M.T. and Dowsett, M. (2010) *Endocr Relat Cancer* 17, R245-62.

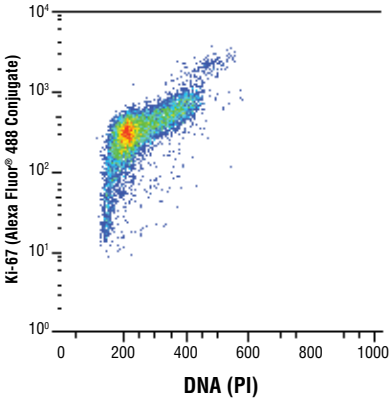
Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. Store at 4°C.
Do not aliquot the antibodies. Protect from light. Do not freeze.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

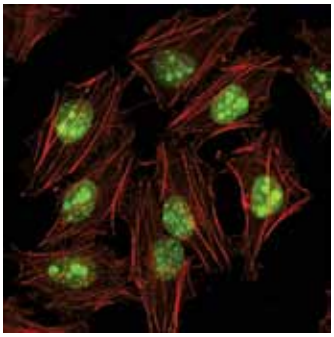


Flow cytometric analysis of Jurkat cells, untreated (blue) or etoposide-treated (green), using ***Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (Alexa Fluor® 488 Conjugate) #9603****.*



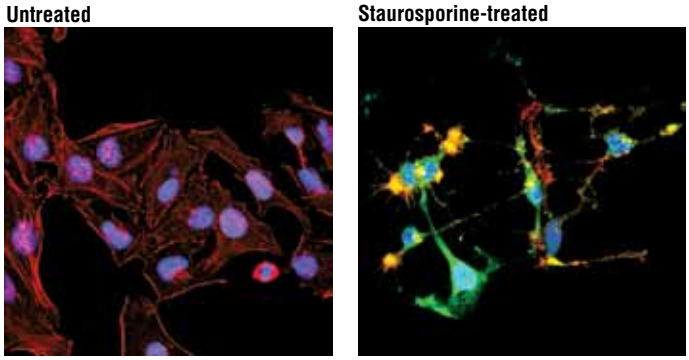
Flow cytometric analysis of Jurkat cells using ***Ki-67 (D3B5) Rabbit mAb (Alexa Fluor® 488 Conjugate) #11882*** *and Propidium Iodide (PI)/RNase Staining Solution #4087 (DNA content).*

HeLa



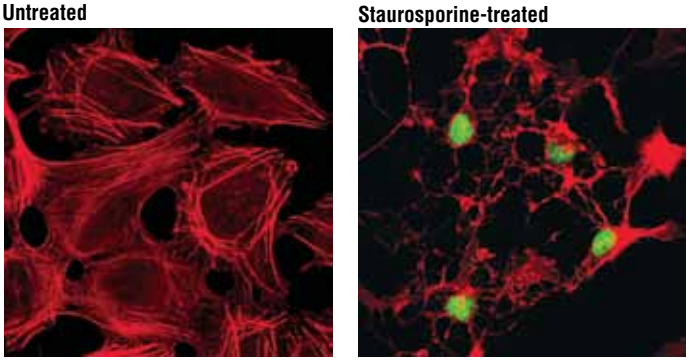
Confocal immunofluorescent analysis of HeLa cells using ***Ki-67 (D3B5) Rabbit mAb (Alexa Fluor® 488 Conjugate) #11882*** *(green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).*

Untreated **Staurosporine-treated**



Confocal immunofluorescent analysis of HeLa cells, untreated (left), or treated with Staurosporine #9953 (right), using ***Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (Alexa Fluor® 488 Conjugate) #9603*** *(green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudo-color = DRAQ5 #4084 (Fluorescent DNA dye).*

Untreated **Staurosporine-treated**



Confocal immunofluorescent analysis of HeLa cells, untreated (left) or treated with Staurosporine #9953 (1 µM, 3 hr; right), using ***Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #9148*** *(green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).*

#12145

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Secondary Antibodies

A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 mL distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at –20°C in 90% methanol.

D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 0.5-1x10^6 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100 µl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
6. Incubate for 30-60 minutes at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in fluorochrome-conjugated secondary antibody*, diluted in Incubation Buffer according to the manufacturer's recommendations.
9. Incubate for 30 minutes at room temperature.
10. Rinse as before in Incubation Buffer by centrifugation.
11. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

*Recommended Secondary Antibodies from Invitrogen.
A-11070 Alexa Fluor® 488 F(ab')2 fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)
A-11017 Alexa Fluor® 488 F(ab')2 fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)