Revision 1

Store at -20C

#8360

Apoptotic Release Antibody Sampler Kit

Cell Signaling TECHNOLOGY®

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Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
Cytochrome c (D18C7) Rabbit mAb	11940	40 µl	14 kDa	Rabbit IgG
Smac/Diablo (79-1-83) Mouse mAb	2954	40 µl	21 kDa	Mouse IgG
HtrA2/Omi (D20A5) Rabbit mAb	9745	40 µl	36 kDa	Rabbit IgG
Caspase-3 (8G10) Rabbit mAb	9665	40 µl	17, 19, 35 kDa	Rabbit IgG
COX IV (4D11-B3-E8) Mouse mAb	11967	40 µl	17 kDa	Mouse IgG1
MEK1/2 (D1A5) Rabbit mAb	8727	40 µl	45 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

Description

The Apoptotic Release Antibody Sampler Kit provides an economical means to evaluate targets that are released from the mitochondria with apoptotic stimuli. The kit contains enough primary antibody to perform four western blots per primary antibody.

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Background

Apoptosis is a regulated physiological process leading to cell death. Caspases, a family of cysteine acid proteases, are central regulators of apoptosis. Initiator caspases (including 8, 9, 10, and 12) are closely coupled to proapoptotic signals. Once activated, these caspases cleave and activate downstream effector caspases (including 3, 6, and 7), which in turn cleave cytoskeletal and nuclear proteins like PARP, α-fodrin, DFF, and lamin A, and induce apoptosis (1).

Cytochrome c is a well conserved electron-transport protein and is part of the respiratory chain localized to the mitochondrial intermembrane space (2). Upon apoptotic stimulation, cytochrome c released from mitochondria associates with procaspase-9 (47 kDa)/Apaf-1. This complex processes caspase-9 from inactive proenzyme to its active form (3). This event further triggers caspase-3 activation and eventually leads to apoptosis (4).

Smac/Diablo is a 21 kDa mammalian mitochondrial protein that functions as a regulatory component during apoptosis (5,6). Upon mitochondrial stress, Smac/Diablo is released from mitochondria and competes with caspases for binding of inhibitor of apoptosis proteins (IAPs) (5,6). The interaction of Smac/Diablo with IAPs relieves the inhibitory effect of the IAPs on caspases (7,8).

High temperature requirement protein A2 (HtrA2)/Omi is a serine protease with homology to the *E. coli* HtrA protein (DegP) and is thought to be involved in apoptosis and stress-induced degradation of misfolded proteins (9). HtrA2 is produced as a 50 kDa zymogen that is cleaved to generate a 36 kDa mature protein that exposes an amino terminal motif (AVPS) resembling that of the IAP inhibitor Smac/Diablo (10-14). Like Smac, interaction between HtrA2 and IAP family members, such as XIAP, antagonizes their inhibition of caspase activity and protection from apoptosis (10-14).

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) (15). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 fragments. Cleavage of caspase-3 requires the aspartic acid residue at the P1 position (16).

Cytochrome c oxidase (COX) is a hetero-oligomeric enzyme consisting of 13 subunits localized to the inner mitochondrial membrane (17-19). It is the terminal enzyme complex in the respiratory chain, catalyzing the reduction of molecular oxygen to water coupled to the translocation of protons across the mitochondrial inner membrane to drive ATP synthesis. The 3 largest subunits forming the catalytic core are encoded by mitochondrial DNA, while the other smaller subunits, including COX IV, are nuclear-encoded. The COX IV (4D11-B3-E8) Mouse mAb can be used effectively as a mitochondrial loading control in cell-based research assays.

MEK1 and MEK2, also called MAPK or Erk kinases, are dual-specificity protein kinases that function in a mitogen activated protein kinase cascade controlling cell growth and differentiation (20-22). Activation

of MEK1 and MEK2 occurs through phosphorylation of two serine residues at positions 217 and 221, located in the activation loop of subdomain VIII, by Raf-like molecules. MEK1/2 is activated by a wide variety of growth factors and cytokines, as well as by membrane depolarization and calcium influx (20-23). MEK activates p44 and p42 MAP kinase by phosphorylating both threonine and tyrosine residues at sites located within the activation loop of kinase subdomain VIII. The MEK1/2 (D1A5) Rabbit mAb can be used effectively as a cytoplasmic loading control in cell-based research assays.

Background References

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