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phospho-Bcl-2 (Ser70) Rabbit pAb

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Catalog # AP52218

Product Information

Application	IHC-P, IHC-F, IF
Reactivity	Human
Predicted	Mouse, Rat, Dog, Rabbit
Host	Rabbit
Clonality	Polyclonal
Calculated MW	26 KDa
Physical State	Liquid
Immunogen	KLH conjugated Synthesised phosphopeptide derived from rat Bcl-2 around the phosphorylation site of Ser70
Epitope Specificity	RT(p-S)PL
Isotype	IgG
Purity	affinity purified by Protein A
Buffer	0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol.
SUBCELLULAR LOCATION	Mitochondrion outer membrane; Single-pass membrane protein. Nucleus membrane; Single-pass membrane protein. Endoplasmic reticulum membrane; Single-pass membrane protein.
SIMILARITY	Belongs to the Bcl-2 family.
SUBUNIT	Forms homodimers, and heterodimers with BAX, BAD, BAK and Bcl-X(L). Heterodimerization with BAX requires intact BH1 and BH2 motifs, and is necessary for anti-apoptotic activity. Interacts with EI24 (By similarity). Also interacts with APAF1, BBC3, BCL2L1, BNIPL, MRPL41 and TP53BP2. Binding to FKBP8 seems to target BCL2 to the mitochondria and probably interferes with the binding of BCL2 to its targets. Interacts with BAG1 in an ATP-dependent manner. Interacts with RAF1 (the 'Ser-338' and 'Ser-339' phosphorylated form). Interacts (via the BH4 domain) with EGLN3; the interaction prevents the formation of the BAX-BCL2 complex and inhibits the anti-apoptotic activity of BCL2. Interacts with G0S2; this interaction also prevents the formation of the anti-apoptotic BAX-BCL2 complex.
Post-translational modifications	Phosphorylation/dephosphorylation on Ser-70 regulates anti-apoptotic activity. Growth factor-stimulated phosphorylation on Ser-70 by PKC is required for the anti-apoptosis activity and occurs during the G2/M phase of the cell cycle. In the absence of growth factors, BCL2 appears to be phosphorylated by other protein kinases such as ERKs and stress-activated kinases. Phosphorylated by MAPK8/JNK1 at Thr-69, Ser-70 and Ser-87, wich stimulates starvation-induced autophagy. Dephosphorylated by protein phosphatase 2A (PP2A). Proteolytically cleaved by caspases during apoptosis. The cleaved protein, lacking the BH4 motif, has pro-apoptotic activity, causes the release of cytochrome c into the cytosol promoting further caspase activity. Monoubiquitinated by PARK2, leading to increase its stability.
DISEASE	Note=A chromosomal aberration involving BCL2 has been found in chronic lymphatic leukemia. Translocation t(14;18)(q32;q21) with immunoglobulin gene regions. BCL2 mutations found in non-Hodgkin lymphomas carrying the chromosomal translocation could be attributed to the Ig somatic

	hypermutation mechanism resulting in nucleotide transitions.
Important Note	This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.
Background Descriptions	The Bcl-2 gene was isolated at the chromosomal breakpoint of t(14;18)-bearing follicular B cell lymphomas(1,2).Bcl-2 blocks cell death following a variety of stimuli and confers a death-sparing effect to certain hematopoietic cell lines following growth factor withdrawal (3,5).Bcl-2 appears to function in several subcellular locations yet lacks any known motifs that would confer insight into its mechanism of action (6,7).A more recently identified protein,designated Bax p21(i.e., Bcl-associated X protein),has extensive amino acid homology with Bcl-2 and both homodimerizes and forms heterodimers with Bcl-2(8). Overexpression of Bax accelerates apoptotic death induced by cytokine deprivation in an IL-3 dependent cell line and Bax also counters the death repressor activty of Bcl-2(8).

Additional Information

Other Names	Bcl-2; Apoptosis regulator Bcl-2; Bcl2
Target/Specificity	Expressed in a variety of tissues.
Dilution	IHC-P=1:100-500,IHC-F=1:100-500,IF=1:100-500,Flow-Cyt=1ug/test
Storage	Store at -20 °C for one year. Avoid repeated freeze/thaw cycles. When reconstituted in sterile pH 7.4 0.01M PBS or diluent of antibody the antibody is stable for at least two weeks at 2-4 °C.

Background

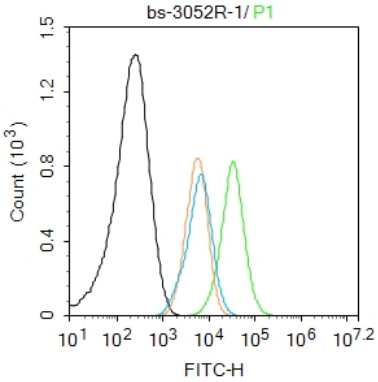
The Bcl-2 gene was isolated at the chromosomal breakpoint of t(14;18)-bearing follicular B cell lymphomas(1,2).Bcl-2 blocks cell death following a variety of stimuli and confers a death-sparing effect to certain hematopoietic cell lines following growth factor withdrawal (3,5).Bcl-2 appears to function in several subcellular locations yet lacks any known motifs that would confer insight into its mechanism of action (6,7).A more recently identified protein,designated Bax p21(i.e., Bcl-associated X protein),has extensive amino acid homology with Bcl-2 and both homodimerizes and forms heterodimers with Bcl-2(8). Overexpression of Bax accelerates apoptotic death induced by cytokine deprivation in an IL-3 dependent cell line and Bax also counters the death repressor activty of Bcl-2(8).

References

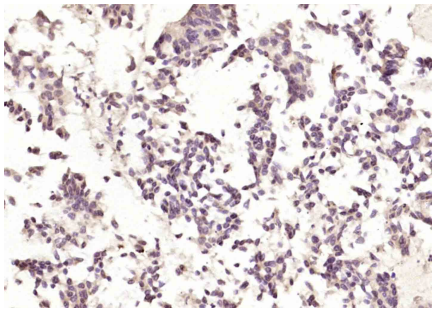
Sato T.,et al.Gene 140:291-292(1994).
Tilly J.L.,et al.Endocrinology 136:232-241(1995).
Castren E.,et al.Neuroscience 61:165-177(1994).
Eliseev R.A.,et al.J. Biol. Chem. 284:9692-9699(2009).

Images

Blank control:HL-60.
Primary Antibody (green line): Rabbit Anti-Phospho-Bcl-2 (Ser70) antibody (AP52218)
Dilution: 1 µg /10^6 cells;
Isotype Control Antibody (orange line): Rabbit IgG .
Secondary Antibody : Goat anti-rabbit IgG-AF488



Dilution: 1 µg /test.
Protocol
The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 0.1%PBST for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.



Paraformaldehyde-fixed, paraffin embedded (human gastric carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-Bcl-2 (Ser70)) Polyclonal Antibody, Unconjugated (AP52218) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.

Citations

- Cytotoxic Activity of Constituent, Inhibits Growth and Migration of HK1 Cells by Inducing Caspase-Dependent Apoptosis and G2/M-Ph