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Caspase 8 Rabbit pAb

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Catalog # AP52053

Product Information

Application	WB, IHC-P, IHC-F, IF
Primary Accession	Q9JHX4
Reactivity	Human, Rat
Predicted	Mouse, Dog, Pig, Horse
Host	Rabbit
Clonality	Polyclonal
Calculated MW	55339
Physical State	Liquid
Immunogen	KLH conjugated synthetic peptide derived from rat Caspase-8 subunit p10 411-482/482
Epitope Specificity	411-482/482
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	Cytoplasm.
SIMILARITY	Belongs to the peptidase C14A family.Contains 2 DED (death effector) domains.
SUBUNIT	Heterotetramer that consists of two anti-parallel arranged heterodimers, each one formed by a 18 kDa (p18) and a 10 kDa (p10) subunit. Interacts with FADD, CFLAR and PEA15. Isoform 9 interacts at the endoplasmic reticulum with a complex containing BCAP31, BAP29, BCL2 and/or BCL2L1. Interacts with TNFAIP8L2.
DISEASE	Defects in CASP8 are the cause of caspase-8 deficiency (CASP8D) [MIM:607271]. CASP8D is a disorder resembling autoimmune lymphoproliferative syndrome (ALPS). It is characterized by lymphadenopathy, splenomegaly, and defective CD95-induced apoptosis of peripheral blood lymphocytes (PBLs). It leads to defects in activation of T-lymphocytes, B-lymphocytes, and natural killer cells leading to immunodeficiency characterized by recurrent sinopulmonary and herpes simplex virus infections and poor responses to immunization.
Important Note	This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.
Background Descriptions	Caspases are cysteine proteases, expressed as inactive precursors, that mediate apoptosis by proteolysis of specific substrates. Caspases have the ability to cleave after aspartic acid residues. There are two classes of caspases involved in apoptosis; initiators (activation by receptor cluster) and effectors (activation by mitochondrial permeability transition). Proapoptotic signals autocatalytically activate initiator caspases, such as Caspase 8 and Caspase 9. Activated initiator caspases then process effector caspases, such as Caspase 3 and Caspase 7, which in turn cause cell collapse.

Additional Information

Gene ID	64044
Other Names	Caspase-8 {ECO:0000303|PubMed:10197541, ECO:0000303|Ref.2}, CASP-8, 3.4.22.61, Casp8 {ECO:0000303|PubMed:10197541, ECO:0000312|RGD:620945}
Target/Specificity	Isoform 1, isoform 5 and isoform 7 are expressed in a wide variety of tissues. Highest expression in peripheral blood leukocytes, spleen, thymus and liver. Barely detectable in brain, testis and skeletal muscle.
Dilution	WB=1:500-2000,IHC-P=1:100-500,IHC-F=1:100-500,IF=1:100-500,Flow-Cyt=1 g /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.

Protein Information

Name	Casp8 {ECO:0000303|PubMed:10197541, ECO:0000312|RGD:620945}
Function	Thiol protease that plays a key role in programmed cell death by acting as a molecular switch for apoptosis, necroptosis and pyroptosis, and is required to prevent tissue damage during embryonic development and adulthood (By similarity). Initiator protease that induces extrinsic apoptosis by mediating cleavage and activation of effector caspases responsible for FAS/CD95-mediated and TNFRSF1A- induced cell death (PubMed:10197541). Cleaves and activates effector caspases CASP3, CASP4, CASP6, CASP7, CASP9 and CASP10 (By similarity). Binding to the adapter molecule FADD recruits it to either receptor FAS/CD95 or TNFRSF1A (PubMed:10197541). The resulting aggregate called the death-inducing signaling complex (DISC) performs CASP8 proteolytic activation (By similarity). The active dimeric enzyme is then liberated from the DISC and free to activate downstream apoptotic proteases (By similarity). Proteolytic fragments of the N-terminal propeptide (termed CAP3, CAP5 and CAP6) are likely retained in the DISC (By similarity). In addition to extrinsic apoptosis, also acts as a negative regulator of necroptosis: acts by cleaving RIPK1 at 'Asp-325', which is crucial to inhibit RIPK1 kinase activity, limiting TNF-induced apoptosis, necroptosis and inflammatory response (By similarity). Also able to initiate pyroptosis by mediating cleavage and activation of gasdermin-C and -D (GSDMC and GSDMD, respectively): gasdermin cleavage promotes release of the N-terminal moiety that binds to membranes and forms pores, triggering pyroptosis (By similarity). Initiates pyroptosis following inactivation of MAP3K7/TAK1 (By similarity). Also acts as a regulator of innate immunity by mediating cleavage and inactivation of N4BP1 downstream of TLR3 or TLR4, thereby promoting cytokine production (By similarity). May participate in the Granzyme B (GZMB) cell death pathways (By similarity). Cleaves PARP1 and PARP2 (By similarity).
Cellular Location	Cytoplasm. Nucleus

Background

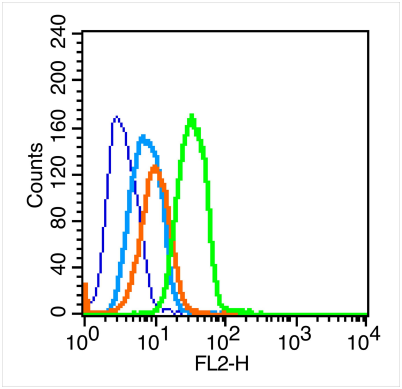
Caspases are cysteine proteases, expressed as inactive precursors, that mediate apoptosis by proteolysis of specific substrates. Caspases have the ability to cleave after aspartic acid residues. There are two classes of caspases involved in apoptosis; initiators (activation by receptor cluster) and effectors (activation by mitochondrial permeability transition). Proapoptotic signals autocatalytically activate initiator caspases, such as Caspase 8 and Caspase 9. Activated initiator caspases then process effector caspases, such as Caspase 3

and Caspase 7, which in turn cause cell collapse.

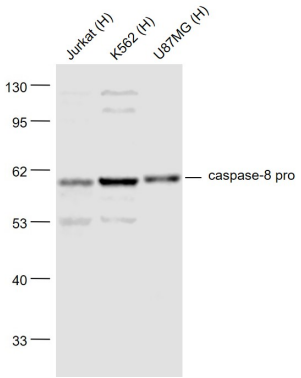
References

Sakamaki K.,et al.Eur. J. Biochem. 253:399-405(1998).
Van de Craen M.,et al.J. Mol. Biol. 284:1017-1026(1998).
Kioschis P.,et al.Submitted (JUL-1997) to the EMBL/GenBank/DDBJ databases.
Milovic-Holm K.,et al.EMBO J. 26:391-401(2007).
Villen J.,et al.Proc. Natl. Acad. Sci. U.S.A. 104:1488-1493(2007).

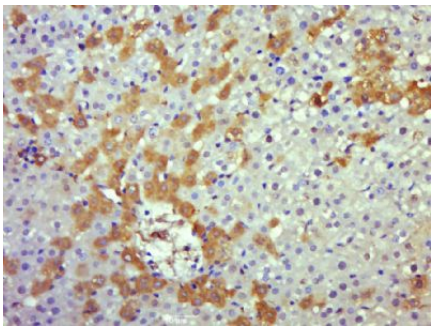
Images



Blank control (blue line): U251 (blue).
Primary Antibody (green line): Rabbit Anti-caspase-8 antibody (AP52053)
Dilution: 1 µg /10^6 cells;
Isotype Control Antibody (orange line): Rabbit IgG .
Secondary Antibody (white blue line): Goat anti-rabbit IgG-PE
Dilution: 1 µg /test.
Protocol
The cells were fixed with 70% ethanol overnight at 4℃ and then permeabilized with 0.1% PBS-Tween for 20 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The cells were then incubated in 1 X PBS/2%BSA/10% goat serum to block non-specific protein-protein interactions followed by the antibody for 15 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.



Sample:
Jurkat(Human) Cell Lysate at 30 ug
K562(Human) Cell Lysate at 30 ug
U87MG(Human) Cell Lysate at 30 ug
Primary: Anti-caspase-8 subunit p18 (AP52053) at 1/1000 dilution
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution
Predicted band size: 55/18 kD
Observed band size: 57 kD



Paraformaldehyde-fixed, paraffin embedded (rat liver); 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 (Caspase 8) Polyclonal Antibody, Unconjugated (AP52053) at 1:500 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.