Technical Data Sheet

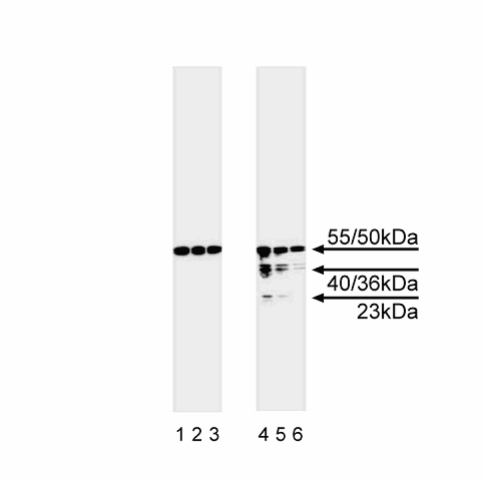
Purified Mouse Anti-Human Caspase-8

Product Information

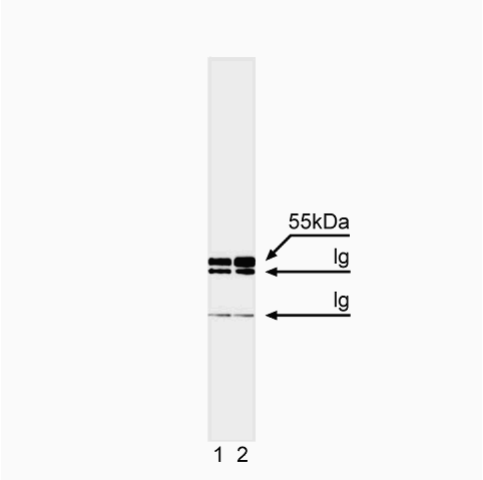
Material Number:	**551242**
Alternate Name:	FLICE, MACH-1, Mch5
Size:	50 µg
Concentration:	0.5 mg/ml
Clone:	3-1-9
Immunogen:	Human Caspase-8 full-length recombinant protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Target MW:	55/50 kDa, 40/36 kDa, 23 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Caspase-8 (FLICE/MACH-1) is a 55 kDa cytosolic protein with homology to the CD95/Fas-associated signal transducer, FADD/MORT-1, as well as to other caspase (ICE/Ced-3) cysteine proteases. The N-terminal region of caspase-8 contains an amino acid sequence, termed the death domain, that facilitates caspase-8-FADD direct interaction. FADD therefore acts as an adapter molecule, allowing caspase-8 to become recruited to the cytoplasmic region of Fas following receptor activation. Viral proteins (v-FLIPS) which inhibit recruitment and activation of caspase-8 have been isolated. Caspase-8 is produced as a proenzyme (55/50 kDa doublet) which upon receptor aggregation is proteolytically cleaved into smaller subunits of 40/36 (doublet), and 23 kDa. Overexpression of caspase-8 is sufficient to induce apoptosis in certain cell lines (e.g., MCF-7) and this phenotype is blocked by overexpression of the caspase-3 protease inhibitor, CrmA. The antibody recognizes both the proform of human caspase-8 (55/50 kDa doublet) as well as the cleaved forms which migrate at 40/36 (doublet) and 23 kDa in SDS/PAGE. It also immunoprecipitates the full-length human caspase-8. Full-length recombinant human caspase-8 protein was used as immunogen.



Western blot analysis of caspase-8. *Lysates from control (lanes 1-3) and camptothecin treated Jurkat cells (lanes 4-6) were probed with anti-human caspase-8 (clone 3-1-9, Cat. No. 551243) at the following concentrations: 0.5 (lanes 1,4), 0.25 (lanes 2,5), and 0.125 µg/ml (lanes 3,6). Caspase-8 is identified as 55/50 kDa (proform), 40/36 kDa (cleaved), and 23 kDa (cleaved) bands in treated cells and the 55 kDa band in control cells.*



Immunoprecipitation/western blot analysis of caspase-8. *Lysates from control (lane 1) or camptothecin-treated Jurkat cells (lane 2) were each immunoprecipitated with anti-human caspase-8 (clone 3-1-9), and western blotted with anti-human caspase-8 (clone 3-1-9). The 55 kDa proform caspase-8 was identified in both control and camptothecin-treated cells.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4°C.

BD Biosciences

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Clone	Catalog Number	Western Blot			Immunoprecipitation		
		55/50kDa	40/36kDa	23kDa	55/50kDa	40/36kDa	23kDa
4-1-20	551244/80851N	+	+	-	-	-	-
B9-2	556466/66231A	+	-	-	-	-	-
Rabbit polyclonal	559932/69236E	+	+	+	-	-	-
3-1-9	551242/80841N	+	+	+	+	-	-
Rabbit polyclonal	552038/8125HE	+	+	+	NT	NT	NT

(+)=positive, (-)=negative, (NT)=not tested

Application Notes

Application

Western blot	Routinely Tested
Immunoprecipitation	Tested During Development

Recommended Assay Procedure:

Applications include western blot analysis (0.125 - 0.5 µg /ml) and immunoprecipitation (4 µg /200µg cell lysate). Jurkat T cells (ATCC CRL-1573) are suggested as a positive control. BD Biosciences Pharmingen offers several caspase-8 antibodies. A Jurkat model cell system was used to evaluate these antibodies; these results are summarized in the following table. However, actual bands observed could vary according to the cell model system or treatment used.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

Boesen-de Cock JG, Tepper AD, de Vries E, van Blitterswijk WJ, Borst J. Common regulation of apoptosis signaling induced by CD95 and the DNA-damaging stimuli etoposide and gamma-radiation downstream from caspase-8 activation. *J Biol Chem.* 1999; 274(20):14255-14261.(Biology)

Cock JG, Tepper AD, de Vries E, van Blitterswijk WJ, Borst J. CD95 (Fas/APO-1) induces ceramide formation and apoptosis in the absence of a functional acid sphingomyelinase. *J Biol Chem.* 1998; 273(13):7560-7565.(Biology)

Muzio M, Chinnaiyan AM, Kischkel FC, et al. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death--inducing signaling complex. *Cell.* 1996; 85(6):817-827.(Biology)

Thome M, Schneider P, Hofmann K, et al. Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. *Nature.* 1997; 386(6624):517-521. (Biology)