

Technical Data Sheet

Purified Mouse Anti-Human Caspase-8

Product Information

Material Number:	551245
Alternate Name:	FLICE, MACH-1, Mch5
Size:	150 µg
Concentration:	0.5 mg/ml
Clone:	4-1-20
Immunogen:	Human caspase-8 recombinant protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
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 form (40/36 kDa doublet) on SDS/PAGE. 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 4-1-20, Cat. No. 551245) at concentrations of: 4.0 (lane 1), 2.0 (lane 2), and 1.0 µg/ml (lane 3). Caspase-8 is identified as 55/50 kDa (proform) and 40/36 kDa (cleaved) bands in treated cells and the 55 kDa in control cells.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. Store undiluted at 4°C. The product contains 3 vials at 50 µg in each vial with the antibody concentration of 0.5 mg/ml.

Application Notes

Application

Western blot	Routinely Tested
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Recommended Assay Procedure:

Applications include western blot analysis (1 - 2 µg/ml). Jurkat T cells (ATCC CRL-1573) are suggested as positive controls.

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.

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

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611451	Jurkat Cell Lysate	500 µg	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/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

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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)