

## Technical Data Sheet

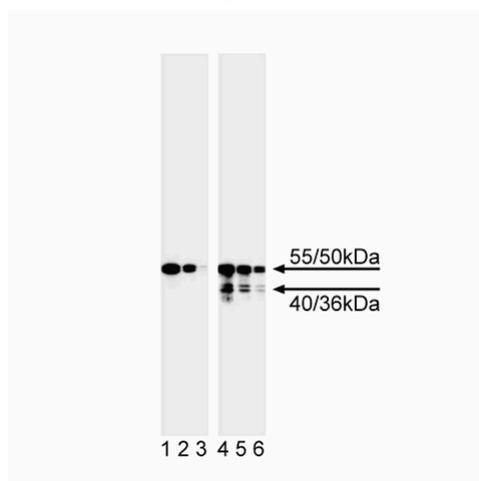
## Purified Mouse Anti-Human Caspase-8

## Product Information

<b>Material Number:</b>	551244
<b>Alternate Name:</b>	FLICE, MACH-1, Mch5
<b>Size:</b>	50 µg
<b>Concentration:</b>	0.5 mg/ml
<b>Clone:</b>	4-1-20
<b>Immunogen:</b>	Human caspase-8 recombinant protein
<b>Isotype:</b>	Mouse IgG1, κ
<b>Reactivity:</b>	QC Testing: Human
<b>Target MW:</b>	55/50 kDa
<b>Storage Buffer:</b>	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.

## 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](http://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

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)

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Fearnhead HO, Rodriguez J, Govek EE, et al. Oncogene-dependent apoptosis is mediated by caspase-9. *Proc Natl Acad Sci U S A.* 1998; 95(23):13664-13669. (Biology)

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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)