

#9748 Store at **-20°C**

Cleaved Caspase-8 (Asp384) (11G10) Mouse mAb

100 µl
 (10 Western mini-blot)



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source	Isotype
W	H	10 kDa	Mouse	IgG1

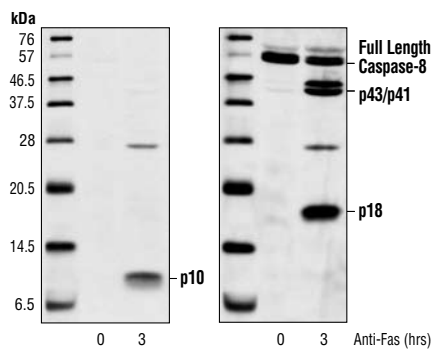
Background: Apoptosis induced through CD95 receptor (Fas/APO-1) and tumor necrosis factor receptor 1 (TNFR1) activates caspase-8 and leads to the release of the caspase-8 active fragments, p18 and p10 (1-3). Activated caspase-8 cleaves and activates downstream effector caspases such as caspase-1, -3, -6 and -7. Caspase-3 ultimately elicits the morphological hallmarks of apoptosis, including DNA fragmentation and cell shrinkage. (This product is sold under license from Promega Corp., U.S. Patent No. 6,350,452.)

Specificity/Sensitivity: Cleaved Caspase-8 (Asp384) (11G10) Mouse mAb detects endogenous levels of the small fragment of caspase-8 resulting from cleavage at aspartic acid 384. The antibody does not cross-react with full length caspase-8.

Source/Purification: Monoclonal antibody is produced by immunizing mice with a synthetic peptide (KLH-coupled) derived from the amino-terminal sequence of p10 of human caspase-8. Antibody is supplied in HEPES buffer with 50% glycerol and less than 0.02% sodium azide.

Selected Application References:
 Cheong, J.W. et al. (2003) Induction of apoptosis by apicidin, a histone deacetylase inhibitor, via the activation of mitochondria-dependent caspase cascades in human Bcr-Abl-positive leukemia cells. *Clin. Cancer Res.* 9, 5018–5027. Application: W.

Background References:
 (1) Muzio, M. et al. (1996) *Cell* 85, 817–827.
 (2) Boldin, M.P. et al. (1996) *Cell* 85, 803–815.
 (3) Fernandes-Alnemri, T. et al. (1996) *Proc. Natl. Acad. Sci. USA* 93, 7464–7469.



Western blot analysis of extracts from SKW6.4 cells, untreated or anti-Fas-treated (1 µg/ml), using Cleaved Caspase-8 (Asp384) (11G10) Mouse mAb (left) or Caspase-8 (1C12) Mouse mAb #9746 (right).

Entrez-Gene ID # 841
Swiss-Prot Acc. # Q14790

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C . Do not aliquot the antibody.

***Species cross-reactivity is determined by Western blot.**

Recommended Antibody Dilutions:
 Western blotting 1:1000

- Companion Products:**
- Biotinylated Protein Ladder #7727
 - BID Antibody (Human Specific) #2002
 - BID (7A3) Mouse mAb (Human Specific) #2006
 - Cleaved Caspase-7 (Asp198) Antibody #9491
 - Caspase-7 Antibody #9492
 - Cleaved Caspase-9 (Asp330) Antibody (Human Specific) #9501
 - Caspase-9 Antibody (Human Specific) #9502
 - Cleaved Caspase-9 (Asp315) Antibody (Human Specific) #9505
 - Cleaved Caspase-3 (Asp175) Antibody #9661
 - Caspase-3 Antibody #9662
 - Caspase-8 (1C12) Mouse mAb #9746
 - Anti-mouse IgG, HRP-linked Antibody #7076
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - 20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.