

CARD11 Antibody

✓ 100 µl
(10 Western mini-blot)

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rev. 05/19/08

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
W	H, M, R	130 kDa	Rabbit

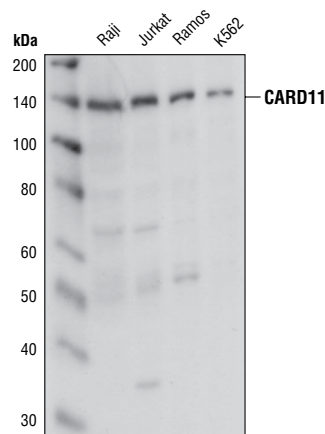
Background: CARD11/Carma1/Bimp3 belongs to the MAGUK (membrane-associated guanylate kinase) family that typically function as molecular scaffolds in the assembly of multiprotein complexes (1,2). The MAGUK family members contain an SH3 domain, a PDZ domain and a GuK domain homologous to guanylate kinase. In addition, CARD11 contains an amino-terminal CARD domain (caspase recruitment domain) functioning in the protein-protein interactions with a number of CARD containing proteins involved in regulating apoptosis and NF-κB activation. CARD11 is predominately expressed in lymphocytes (1,2). It associates with the CARD domain of Bcl10 and when overexpressed leads to the phosphorylation of Bcl10 and activation of NF-κB (1,2). CARD11, is constitutively associated with lipid rafts, and is thought to function by recruiting Bcl10 and MALT1 and triggering the phosphorylation of IKKs (3,7). Several studies using genetic disruption of CARD11 or dominant-negative mutations have demonstrated that it plays a critical role in NF-κB activation and lymphocyte signaling (4–7).

Specificity/Sensitivity: CARD11 Antibody detects endogenous levels of total CARD11 protein. Cross reactivity was not detected with other family members at physiological conditions.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues surrounding methionine 362 of human CARD11. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- (1) Bertin, J. et al. (2001) *J. Biol. Chem.* 276, 11877–82.
- (2) Gaide, O. et al. (2001) *FEBS Lett.* 496, 121–7.
- (3) Stilo, R. et al. (2004) *J. Biol. Chem.* 279, 34323–31.
- (4) Jun, J.E. et al. (2003) *Immunity* 18, 751–62.
- (5) Hara, H. et al. (2003) *Immunity* 18, 763–75.
- (6) Wang, D. et al. (2002) *Nat. Immunol.* 3, 830–5.
- (7) Gaide, O. et al. (2002) *Nat. Immunol.* 3, 836–43.



Western blot analysis of extracts from Raji, Jurkat, Ramos and K562 cells, using CARD11 Antibody.

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

Recommended Antibody Dilutions:

Western blotting 1:1000

Companion Products:

Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb #3033
Phospho-IκB-α (Ser32/36) (5A5) Mouse mAb #9246
Bcl10 Antibody #4246
NF-κB p65 Antibody #3034
Phototope-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
Anti-rabbit IgG, HRP-linked Antibody #7074
Prestained Protein Marker, Broad Range (Premixed Format) #7720
Biotinylated Protein Ladder Detection Pack #7727
20X LumiGLO® Reagent and 20X Peroxide #7003

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

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish B—bovine

Dg—Dog Pg—Pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 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)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 μ l Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) 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 μ l per well of 6-well plate or 500 μ l 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 μ l sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 μ l onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 μ l/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 μ l/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.