

#3182 Store at -20°C

# CrkL (32H4) Mouse mAb



✓ 100 µl  
(10 Western mini-blot)

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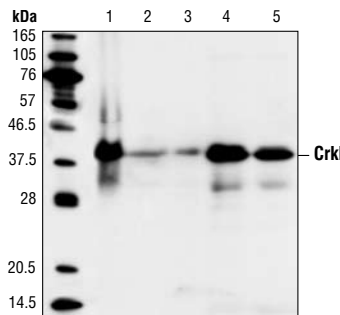
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Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, R, Hm	39 kDa	Mouse

**Background:** CrkL, a 39 kDa adaptor protein, has a key regulatory role in hematopoietic cells. CrkL has one SH2 and two SH3 domains, with 60% homology to CrkII (1). The amino-terminal SH3 domain of CrkL binds proteins such as C3G, SOS, PI3K, c-Abl and BCR/Abl. The SH2 domain of CrkL can bind to tyrosine-phosphorylated proteins such as Cbl, HEF1, CAS and paxillin (2,3). CrkL is involved in various signaling cascades initiated by different cytokines and growth factors. The biological outcomes of the Crk-activated signal transduction include the modulation of cell adhesion, cell migration and immune cell responses (4). CrkL is a prominent substrate of the BCR/Abl oncoprotein in chronic myelogenous leukemia and binds to both BCR/Abl and c-Abl (5). CrkL is prominently and constitutively tyrosine phosphorylated in CML neutrophils and is not phosphorylated in normal neutrophils. Moreover, stimulation of normal neutrophils with cytokines and agonists does not induce tyrosine phosphorylation of this protein (6), indicating that it may be a useful target for therapeutic intervention or as a disease marker. Tyr207 in CrkL is the BCR/Abl phosphorylation site (7).

**Specificity/Sensitivity:** CrkL (32H4) Mouse mAb detects endogenous levels of CrkL protein. It does not cross-react with related proteins.

**Source/Purification:** Monoclonal antibody is produced by immunizing mice with a synthetic peptide (KLH-coupled) corresponding to amino-terminal residues of human CrkL.



Western blot analysis of extracts from HT29 (lane 1), THP1 (lane 2), C6 (lane 3), SUP-M2 (lane 4) and Jurkat (lane 5) cells using CrkL (32H4) Mouse mAb.

### Background References:

- (1) Satter, M. and Salgia, R. (1998) *Leukemia* 12, 637-644.
- (2) Feller, S.M. et al. (1998) *J. Cell. Physiol.* 177, 535-552.
- (3) Kiyokawa, E. et al. (1997) *Crit. Rev. Oncog.* 8, 329-342.
- (4) Feller, S.M. et al. (2001) *Oncogene* 20, 6348-6371.
- (5) Grumbach, I.M. et al. (2001) *Br. J. Haematol.* 112, 327-336.
- (6) Nicholas, G.L. et al. (1994) *Blood* 84, 2912-2918.
- (7) de Jong, R. et al. (1997) *Oncogene* 14, 507-513.

Entrez-Gene ID # 1399  
Swiss-Prot Acc. # P46109

**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:**  
Phospho-CrkL (Tyr207) Antibody #3181  
Anti-mouse IgG, HRP-linked Antibody #7076  
Prestained Protein Marker, Broad Range (Premixed Format) #7720  
Biotinylated Protein Ladder #7727  
20X LumiGLO® Reagent and 20X Peroxide #7003

**IMPORTANT: 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.**

**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  
 F—Flow cytometry E—ELISA D—DELFIATM  
 Z—zebra fish B—bovine All—all species expected  
 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<sup>®</sup>-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<sup>®</sup> 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 cm<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.