

# MCF2/Dbl Antibody

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.

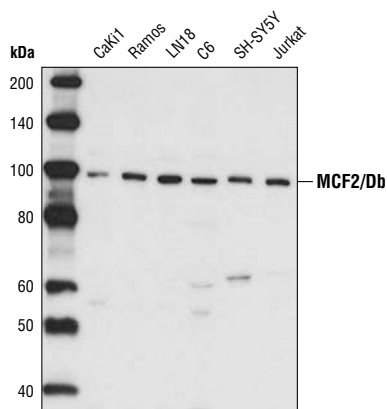
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, R	100 kDa	Rabbit

**Background:** The MCF2/Dbl proto-oncogene product is the founding member of the Dbl family of Rho guanine nucleotide exchange factors (GEFs) that are characterized by their Dbl homology (DH) domain (1). GEFs stimulate the formation of the active, GTP-bound form of small GTPases such as Rho, Rac and Cdc42, signaling to various downstream molecules and regulating diverse cell functions. While the overexpressed, full-length Dbl gene has transforming activity (2), mutations resulting in truncated Dbl cause the protein to become highly oncogenic. This truncated form of Dbl, which lacks the amino-terminal 497 amino acids, has constitutive GEF activity (3) and is more stable than the full-length variant (4), allowing for increased signaling to downstream effector molecules.

Dbl interacts with ezrin, a member of the ezrin/radixin/moesin (ERM) family of proteins that links the plasma membrane to the actin cytoskeleton. Dbl interacts with ezrin in lipid microdomains, which leads to Cdc42 activation and the regulation of processes such as filopodia formation and cell polarity (5,6). Dbl localization and biological activities are regulated in part by phosphatidylinositol 3-kinase (PI3K) (7). Dbl is also involved in cell survival and inhibits apoptosis through induction of Akt phosphorylation at Thr308 (8).

**Specificity/Sensitivity:** MCF2/Dbl Antibody recognizes endogenous levels of total MCF2/Dbl protein. Based on amino acid sequence homology, the antibody is expected to recognize splice variants 1-4 of human MCF2/Dbl. The antibody is not expected to recognize the onco-Dbl protein formed through amino-terminal truncation.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to the amino terminus of human MCF2/Dbl. Antibodies are purified using protein A and peptide affinity chromatography.



Western blot analysis of extracts from various cell types using MCF2/Dbl Antibody.

**Background References:**

- (1) Zheng, Y. (2001) *Trends Biochem Sci* 26, 724–32.
- (2) Ron, D. et al. (1988) *EMBO J* 7, 2465–73.
- (3) Hart, M.J. et al. (1991) *Nature* 354, 311–4.
- (4) Kamynina, E. et al. (2007) *Mol Cell Biol* 27, 1809–22.
- (5) Batchelor, C.L. et al. (2007) *Cell Cycle* 6, 353–63.
- (6) Prag, S. et al. (2007) *Mol Biol Cell* 18, 2935–48.
- (7) Vanni, C. et al. (2006) *Cell Cycle* 5, 2657–65.
- (8) Morley, S. et al. (2007) *Cell Signal* 19, 211–8.

**Entrez-Gene ID** #4168  
**Swiss-Prot Acc.** #P10911

**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.*

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

**Recommended Antibody Dilutions:**

Western blotting 1:1000

**Companion Products:**

- Cdc42 (11A11) Rabbit mAb #2466
- Phospho-Rac1/cdc42 (Ser71) Antibody #2461
- Rac1/2/3 (L129) Antibody #2467
- RhoA (67B9) Rabbit mAb #2117
- RhoB Antibody #2098
- 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 nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

## Western Immunoblotting Protocol (Primary Antibody Incubation in Nonfat Dry 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)
- Bovine Serum Albumin (BSA)
- 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 #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 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 markers (#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® (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.