

#3254 Store at -20°C

# DR3 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.

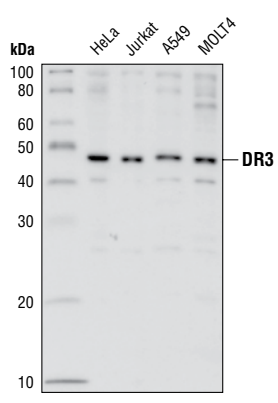
Applications	Species Cross-Reactivity	Molecular Wt.	Source
W	H	48 kDa	Rabbit

**Background:** The tumor necrosis factor receptor family, which includes TNF-R1, Fas, DR3, DR4, DR5 and DR6, plays an important role in the regulation of apoptosis in various physiological systems (1,2). The receptors are activated by a family of cytokines that include TNF, FasL and TRAIL. They are characterized by a highly conserved extracellular region containing cysteine-rich repeats and a conserved intracellular region of about 80 amino acids termed the death domain (DD). The DD is important for transducing the death signal by recruiting other DD containing adaptor proteins (FADD, TRADD, RIP) to the death-inducing signaling complex (DISC) resulting in activation of caspases.

DR3/WSL-1/Apo-3/TRAMP/LARD is a TNFR family member containing the characteristic extracellular cysteine-repeats, transmembrane region, and intracellular DD (3-7). DR3 is activated by its ligand Apo-3L/TWEAK to induce apoptosis and activation of NF-κB (8,9). Like TNF-R1, DR3 binds to the DD adaptor protein TRADD, which can then associate with other DD proteins like FADD and RIP as well as members of the TRAF family (3,4). Tissue expression of DR3 is very restricted, primarily seen on the surface of activated thymocytes and lymphocytes and plays an important role in thymocyte negative selection (3,4,10). Studies have also indicated an association with DR3 and rheumatoid arthritis (11,12).

**Specificity/Sensitivity:** DR3 Antibody detects endogenous levels of human DR3. It is not expected to cross-react with other TNF receptor family members.

**Source/Purification:** DR3 Antibody is produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues within the intracellular domain of human DR3. Antibodies were purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from various cell lines, using DR3 Antibody.

### Background References:

- (1) Nagata, S. (1997) *Cell* 88, 355-365.
- (2) Thorburn, A. (2004) *Cell Signal* 16, 139-144.
- (3) Chinnaiyan, A.M. et al. (1996) *Science* 274, 990-992.
- (4) Kitson, J. et al. (1996) *Nature* 384, 372-375.
- (5) Marsters, S.A. et al. (1996) *Curr. Biol.* 6, 1669-1676.
- (6) Bodmer, J.L. et al. (1997) *Immunity* 6, 79-88.
- (7) Screaton, G.R. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 4615-4619.
- (8) Marsters, S.A. et al. (1998) *Curr. Biol.* 8, 525-528.
- (9) Kaptein, A. et al. (2000) *FEBS Lett.* 485, 135-141.
- (10) Wang, E.C. et al. (2001) *Mol. Cell Biol.* 21, 3451-3461.
- (11) Osawa, K. et al. (2004) *Genes Immun.* 5, 439-443.
- (12) Borysenko, C.W. et al. (2005) *Biochem. Biophys. Res. Commun.* 328, 794-799.

**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:

- DR5 Antibody #3696
- FasL Antibody #4273
- FADD Antibody (Human Specific) #2782
- TRADD (7G8) Rabbit mAb #3684
- RIP Antibody #4926
- TWEAK Receptor/Fn14 Antibody #4403
- 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 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 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 µl Tween-20 (100%).
- Phototope<sup>®</sup>-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<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 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<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.