

#3736 Store at -20°C

# TNF-R1 (C25C1) Rabbit mAb



✓ 100 µl  
(10 Western mini-blots)

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New 10/07

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

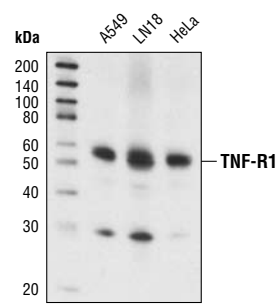
Applications W, IP	Species Cross-Reactivity* H	Molecular Wt. 55 kDa	Source Rabbit**	Isotype IgG
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**Background:** TNF-α is an important cytokine produced by numerous cell types including neutrophils, activated lymphocytes, macrophages and NK cells. It plays a critical role in inflammatory responses and in apoptosis (1). TNF-α exists as a membrane-anchored and soluble form, both of which show biological activity. Response to TNF-α is mediated through two receptors, TNF-R1, which is widely expressed, and TNF-R2, which is expressed mainly in immune and endothelial cells (2). Antagonists to TNF-α have been validated as therapeutic targets for rheumatoid arthritis and other immune disorders (3).

The two receptors for TNF-α, TNF-R1 (55 kDa) and TNF-R2 (75 kDa) can mediate distinct cellular responses (4,5). In most cases cytotoxicity elicited by TNF has been reported to act through TNF-R1 (6,7). Cytotoxicity is mediated by a "death domain" with the intracellular region of the receptor that binds to the death domain adaptor protein TRADD and triggers the activation of caspases (8). Soluble forms of both receptors have also been characterized which can bind TNF-α and may play an important role in immune disorders (9,10).

**Specificity/Sensitivity:** TNF-R1 (C25C1) Rabbit mAb detects endogenous levels of human TNF-R1. This antibody does not appear to cross-react with other related family members.

**Source/Purification:** Monoclonal antibody is produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues within the intracellular region and surrounding Ser331 of human TNF-R1 .



Western blot analysis of extracts from A549, LN18 and HeLa cells using TNF-R1 (C25C1) Rabbit mAb.

### Background References:

- (1) Aggarwal, B.B. (2003) *Nat Rev Immunol* 3, 745–56.
- (2) Locksley, R.M. et al. (2001) *Cell* 104, 487–501.
- (3) Taylor, P.C. et al. (2004) *Curr Opin Biotechnol* 15, 557–63.
- (4) Tartaglia, L.A. et al. (1991) *Proc Natl Acad Sci USA* 88, 9292–6.
- (5) Peschon, J.J. et al. (1998) *J Immunol* 160, 943–52.
- (6) Tartaglia, L.A. et al. (1993) *Cell* 73, 213–6.
- (7) Rothe, J. et al. (1993) *Nature* 364, 798–802.
- (8) Chen, G. and Goeddel, D.V. (2002) *Science* 296, 1634–5.
- (9) Humbert, M. et al. (1994) *Am J Respir Crit Care Med* 149, 1681–5.
- (10) Schröder, J. et al. *Infection* 23, 143–8.

Entrez-Gene ID #7132  
Swiss-Prot Acc. #P19438

**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.**  
**\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.**

**Recommended Antibody Dilutions:**  
Western Blotting 1:1000  
Immunoprecipitation 1:100

**Companion Products:**  
Tumor Necrosis Factor-α #2169  
TNF-α Antibody #3707  
TNF-R2 Antibody #3727  
DR3 Antibody #3254  
DR5 Antibody #3696  
DcR1 Antibody #4756  
DcR3 Antibody #4758  
TRADD (7G8) Rabbit mAb #3684  
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 #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

## Western Immunoblotting Protocol (Primary Antibody 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®-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.

## Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

### A Solutions and Reagents

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

1. 1X Phosphate Buffered Saline (PBS)
2. **1X Cell Lysis Buffer:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM  $\beta$ -glycerophosphate, 1 mM  $\text{Na}_3\text{VO}_4$ , 1  $\mu\text{g}/\text{ml}$  Leupeptin

**NOTE:** Add 1 mM PMSF immediately prior to use.

1. **Transfer Buffer:** 25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
2. **Protein A or G Agarose Beads:** (Can be stored for 2 weeks at 4°C.) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
3. **3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

### B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer to each plate (10 cm) and incubate the plates on ice for 5 minutes.
4. Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate samples on ice three times for 5 seconds each.
6. Microcentrifuge for 10 minutes at 14,000 X g, 4°C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at -80°C.

### C Immunoprecipitation

Optional: It may be necessary to perform a lysate pre-clearing step to reduce non-specific binding to the Protein A/G agarose beads (See section below).

1. Take 200  $\mu\text{l}$  cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
2. Add either protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500  $\mu\text{l}$  of 1X cell lysis buffer. Keep on ice during washes.
4. Resuspend the pellet with 20  $\mu\text{l}$  3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes and microcentrifuge for 1 minute at 14,000 X g.
6. Load the sample (15–30  $\mu\text{l}$ ) on SDS-PAGE gel (12–15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

### Cell Lysate Pre-Clearing (Optional)

1. Take 200  $\mu\text{l}$  cell lysate and add to either Protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry).
2. Incubate at 4°C for 30 – 60 minutes.
3. Spin for 10 minutes at 4°C. Transfer the supernatant to a fresh tube.
4. Proceed to step 1 of Immunoprecipitation.