

#2219 Store at -20°C

Toll-like Receptor 4 Antibody (Rodent Specific)

✓ 100 µl (10 Western mini-blots)



Orders ■ 877-616-CELL (2355) orders@cellsignal.com
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New 02/08

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

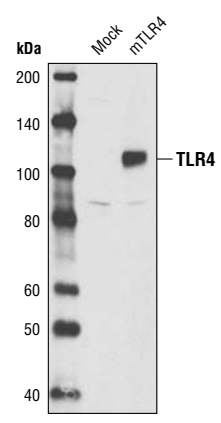
Applications W Transfected	Species Cross-Reactivity* M, (R)	Molecular Wt. 110 kDa	Source Rabbit
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Background: Members of the Toll-like receptor (TLR) family, named for the closely related Toll receptor in *Drosophila*, play a pivotal role in innate immune responses (1-3). TLRs recognize conserved motifs found in various pathogens and mediate defense responses. Triggering of the TLR pathway leads to the activation of NF-κB and subsequent regulation of immune and inflammatory genes. The TLRs and members of the IL-1 receptor family share a conserved stretch of approximately 200 amino acids known as the TIR domain. Upon activation, TLRs associate with a number of cytoplasmic adaptor proteins containing TIR domains including MyD88 (myeloid differentiation factor), MAL/TIRAP (MyD88-adaptor-like/TIR-associated protein), TRIF (Toll-receptor-associated activator of interferon) and TRAM (Toll-receptor associated molecule). This association leads to the recruitment and activation of IRAK1 and IRAK4, which form a complex with TRAF6 to activate TAK1 and IKK. Activation of IKK leads to the degradation of IκB that normally maintains NF-κB inactivity by sequestering it in the cytoplasm.

TLR4 functions in association with MD-2 in the recognition and initiation of immune responses elicited by lipopolysaccharide (LPS) of Gram-negative bacteria (4-8). TLR4 triggers the activation of NF-κB, IRF-3, and MAPK pathways leading to the production of inflammatory cytokines (9).

Specificity/Sensitivity: Toll-like Receptor 4 Antibody (Rodent Specific) detects transfected levels of total TLR4 protein. Cross reactivity was not detected with other TLR family members.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Cys549 within the extracellular region of mouse and rat TLR4. Antibodies were purified by peptide affinity chromatography.



Western blot analysis of extracts from HeLa cells, mock transfected or transfected with mouse TLR4, using Toll-like Receptor 4 Antibody (Rodent Specific).

Background References:

- (1) Akira, S. (2003) *J Biol Chem* 278, 38105-8.
- (2) Beutler, B. (2004) *Nature* 430, 257-63.
- (3) Dunne, A. and O'Neill, L.A. (2003) *Sci STKE* 2003, re3.
- (4) Rock, F.L. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 588-593.
- (5) Poltorak, A. et al. (1998) *Science* 282, 2085-2088.
- (6) Chow, J.C. et al. (1999) *J. Biol. Chem.* 274, 10689-10692.
- (7) Hoshino, K. et al. (1999) *J. Immunol.* 162, 3749-3752.
- (8) Shimazu, R. et al. (1999) *J. Exp. Med.* 189, 1777-1782.
- (9) Kawai, T. and Akira, S. (2006) *Cell Death Differ.* 13, 816-825.

Entrez-Gene ID # 7099
Swiss-Prot Acc. # O00206

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:

- Toll-like Receptor 2 Antibody #2229
- Toll-like Receptor 3 Antibody #2253
- Toll-like Receptor 7 Antibody #2633
- Toll-like Receptor 9 Antibody #2254
- MyD88 Antibody #3699
- Phospho-IRF-3 (Ser396) (4D4G) Rabbit mAb #4947
- Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb #3033
- NF-κB p65 (C22B4) Rabbit mAb #4764
- 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 F—Flow cytometry E—ELISA D—DELFIATM
 Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus 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 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 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.