Store at -20°C

# #48697

# Toll-like Receptor Antibody Sampler Kit II

Cell Signaling TECHNOLOGY®

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New 06/19

**For Research Use Only. Not For Use In Diagnostic Procedures.**

| Products Included | Product # | Quantity | Mol. Wt. | Isotype/Source |
|---|---|---|---|---|
| Toll-like Receptor 1 Antibody | 2209 | 20 µl | 86 kDa | Rabbit |
| Toll-like Receptor 2 (D7G9Z) Rabbit mAb | 12276 | 20 µl | 90-105 kDa | Rabbit IgG |
| Toll-like Receptor 3 (D10F10) Rabbit mAb | 6961 | 20 µl | 115-130 kDa | Rabbit IgG |
| Toll-like Receptor 6 (D1Z8B) Rabbit mAb | 12717 | 20 µl | 90-110 kDa | Rabbit IgG |
| Toll-like Receptor 7 (D7) Rabbit mAb | 5632 | 20 µl | 140 kDa | Rabbit IgG |
| Toll-like Receptor 8 (D3Z6J) Rabbit mAb | 11886 | 20 µl | 150 kDa | Rabbit IgG |
| Toll-like Receptor 9 (D9M9H) XP® Rabbit mAb | 13674 | 20 µl | 130 kDa | Rabbit IgG |
| Anti-rabbit IgG, HRP-linked Antibody | 7074 | 100 µl | | Goat |

**See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.**

**Description:** The Toll-like Receptor Antibody Sampler Kit II provides an economical means of detecting expression of various Toll-like receptors (TLRs). The kit contains enough primary and secondary antibodies to perform at least two western blot experiments.

**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 (1-4). TLRs recognize conserved motifs found in various pathogens and mediate defense responses (5-7). TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11 are localized to the plasma membrane, while TLR3, TLR7, TLR8, and TLR9 are localized to intracellular membranes including endosomal membranes. Triggering of the TLR pathway leads to the activation of NF-κB and subsequent regulation of immune and inflammatory genes (4). The TLRs and members of the IL-1 receptor family share a conserved stretch of approximately 200 amino acids known as the Toll/Interleukin-1 receptor (TIR) domain (1). Upon activation, TLRs associate with a number of cytoplasmic adaptor proteins containing TIR domains, including myeloid differentiation factor 88 (MyD88), MyD88-adaptor-like/TIR-associated protein (MAL/TIRAP), Toll-receptor-associated activator of interferon (TRIF), and Toll-receptor-associated molecule (TRAM) (8-10). This association leads to the recruitment and activation of IRAK1 and IRAK4, which form a complex with TRAF6 to activate TAK1 and IKK (8,11-14). Activation of IKK leads to the degradation of IκB, which normally maintains NF-κB in an inactive state by sequestering it in the cytoplasm. TLR1 and TLR6 associate with TLR2 to cooperatively mediate response to bacterial lipoproteins and fungal zymosan (6,15). TLR3 is an endosomal TLR that recognizes double-stranded RNA derived from viruses (7). TLR7 and TLR8 recognize single-stranded viral RNA and are also activated by synthetic imidazoquinoline compounds including R-848 (16,17). TLR9 recognizes unmethylated CpG motifs present on bacterial DNA (18).

**Specificity/Sensitivity:** Each antibody in the Toll-like Receptor Antibody Sampler Kit II detects endogenous levels of its target protein. Toll-like Receptor 3 (D10F10) Rabbit mAb detects a 75 kDa protein of unknown origin in some cell lines and tissues. Toll-like Receptor 6 (D1Z8B) Rabbit mAb detects a 72 kDa protein of unknown origin. Toll-like Receptor 6 (D1Z8B) Rabbit mAb is not approved for IP in mouse samples. Toll-like Receptor 8 (D3Z6J) Rabbit mAb detects 30 kDa and 37 kDa proteins of unknown origin. Toll-like Receptor 9 (D9M9H) XP® Rabbit mAb is predicted to react with known full-length isoforms of TLR9, but not with the cleaved TLR9 protein.

**Source/Purification:** Monoclonal antibodies are produced by immunizing rabbits with synthetic peptides corresponding to residues surrounding Val303 of human TLR2 protein, Val495 of human TLR3 protein, Pro47 of human TLR6 protein, Pro141 of human TLR8 protein, Pro450 of human TLR9 protein, or recombinant protein specific to the leucine-rich repeats within human TLR7 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues at the amino terminus of human TLR1 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

**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.*

**Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.**

**Background References:**

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**Applications:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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# Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
**NOTE:** Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

## A. Solutions and Reagents

**NOTE:** Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml $dH_2O$, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml $dH_2O$, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with $dH_2O$.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml $dH_2O$, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml $dH_2O$, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml $dH_2O$, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

## B. Protein Blotting

### A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

## C. Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 $cm^2$) of membrane; for different sized membranes, adjust volumes accordingly.

### I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

### II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

## D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.