abcam

ab13556

Anti-TLR4 antibody

Anti-TLR4 antibody (ab13556) is a rabbit polyclonal antibody detecting TLR4 in Western Blot, Flow Cytometry, Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Suitable for Human, Mouse.- Over 330 publications- Trusted since 2004

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Preservative: 0.09% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine)
Form	Liquid
Clonality	Polyclonal
Immunogen	Synthetic Peptide within Human TLR4 aa 400-450. The exact immunogen used to generate this antibody is proprietary information. Database link O00206
Purification technique	Affinity purification Protein A
Specificity	TLR4 expression levels and cleavage or degradation products can vary between different cell and tissue samples. Customers have observed this variability in WB band size and our laboratory has confirmed this variability as well observing lower molecular weight cleavage and degradation products and in some samples a lack of the full length TLR4 band. The TLR4 cleavage and degradation products and potential lack of full length TLR4 are well documented in the literature, including PMID 16885150 and 22927440. We recommend running a positive control human intestine tissue lysate. We have obtained both positive and negative feedback from researchers using this antibody with rat samples (see Abreviews). Due to the inconsistency, we have removed rat as a guaranteed species and welcome any further feedback from researchers using this antibody.
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	Block in 5% Skim Milk in 1X TBST. Other potential band(s): ~130kDa (TLR4+MD-2). We suggest use TLR4 overexpression 293T lysate (whole cell) (ab94063) as positive control.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	-
Notes	-

Not recommended

Species	Human
Dilution info	-
Notes	-

IHC-Fr

Not recommended

Species	Human, Mouse
Dilution info	-
Notes	-

Target data

See full target information TLR4

Function

Transmembrane receptor that functions as a pattern recognition receptor recognizing pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) to induce innate immune responses via downstream signaling pathways (PubMed:10835634, PubMed:15809303, PubMed:16622205, PubMed:17292937, PubMed:17478729, PubMed:20037584, PubMed:20711192, PubMed:23880187, PubMed:27022195, PubMed:29038465, PubMed:17803912, PubMed:15852007). At the plasma membrane, cooperates with LY96 to mediate the innate immune response to bacterial lipopolysaccharide (LPS) (PubMed:27022195). Also involved in LPS-independent inflammatory responses triggered by free fatty acids, such as palmitate, and Ni(2+) (PubMed:20711192). Mechanistically, acts via MYD88, TIRAP and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response (PubMed:10835634, PubMed:21393102, PubMed:27022195, PubMed:36945827, PubMed:9237759). Alternatively, CD14-mediated TLR4 internalization via endocytosis is associated with the initiation of a MYD88-independent signaling via the TICAM1-TBK1-IRF3 axis leading to type I interferon production (PubMed:14517278). In addition to the secretion of proinflammatory cytokines, initiates the activation of NLRP3 inflammasome and formation of a positive feedback loop between autophagy and NF-kappa-B signaling cascade (PubMed:32894580). In complex with TLR6, promotes inflammation in monocytes/macrophages by associating with TLR6 and the receptor CD86 (PubMed:23880187). Upon ligand binding, such as oxLDL or amyloid-beta 42, the TLR4:TLR6 complex is internalized and triggers inflammatory response, leading to NF-kappa-B-dependent production of CXCL1, CXCL2 and CCL9 cytokines, via MYD88 signaling pathway, and CCL5 cytokine, via TICAM1 signaling pathway (PubMed:23880187). In myeloid dendritic cells, vesicular stomatitis virus glycoprotein G but not LPS promotes the activation of IRF7, leading to type I IFN production in a CD14-dependent manner (PubMed:15265881, PubMed:23880187).

Required for the migration-promoting effects of ZG16B/PAUF on pancreatic cancer cells.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Product SpecificationsAnti-TLR4 antibody (ab13556) is a rabbit polyclonal antibody and is validated for use in Flow Cyt, WB, IHC-P in human and mouse samples.Quality and ValidationAnti-TLR4 antibody (ab13556) specifically detects TLR4 (UniProt ID: O00206; Molecular weight: 93kDa) and is sold in 100 µg selling sizes.Abcam's high quality validation processes ensure Anti-TLR4 antibody (ab13556) has high sensitivity and specificity.Anti-TLR4 antibody (ab13556) has been cited over 335 times in peer reviewed journals and is trusted by the scientific community.Anti-TLR4 antibody (ab13556) has 19 independent reviews from customers.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Toll-like receptor 4 (TLR4) is a membrane protein involved in the innate immune response. It serves as a receptor that recognizes pathogen-associated molecular patterns such as lipopolysaccharides from bacterial cell walls. Often referred to by its alternate names TLR-2 and CD284 this protein has a molecular weight of approximately 95 kDa. TLR4 is mainly expressed on the surface of immune cells like macrophages and dendritic cells enabling these cells to detect and respond to microbial invaders effectively.
Biological function summary	TLR4 plays an important role in activating the immune system. It forms a complex with myeloid differentiation factor 2 (MD-2) to facilitate binding to its ligands. Upon activation TLR4 triggers a signaling cascade that leads to the release of pro-inflammatory cytokines and interferons which are important for initiating the immune response. This early detection mechanism alerts the immune system to the presence of pathogens allowing the body to mount a defense.

Pathways	TLR4 participates in the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. These pathways are central to the inflammatory response with TLR4 acting as a sensor that activates downstream components like the MyD88 and TRIF proteins. These interactions result in the expression of genes that produce cytokines and enzymes involved in inflammation and immunity.
Associated diseases and disorders	TLR4 has been implicated in the development of sepsis and atherosclerosis. In sepsis TLR4 recognizes lipopolysaccharides from bacterial infections which can cause excessive inflammation if uncontrolled. In atherosclerosis TLR4 receptors on vascular cells respond to endogenous ligands such as oxidized low-density lipoproteins contributing to plaque formation. The TLR4 interaction with proteins like CD14 and MD-2 is significant as it influences the inflammatory pathways that exacerbate these conditions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

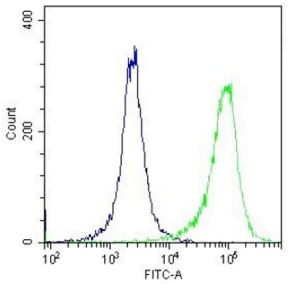
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

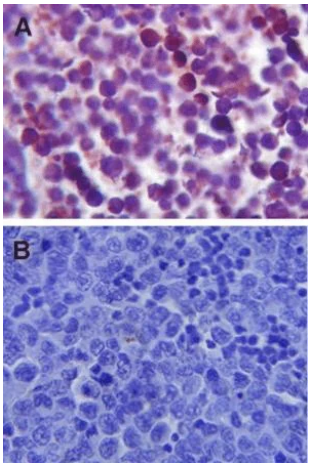
8 product images



Flow Cytometry - Anti-TLR4 antibody (ab13556)

TLR4 Flow Cytometry staining using rabbit Anti-TLR4 antibody

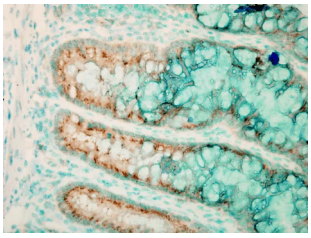
Flow cytometry analysis of THP-1 (Human monocytic leukemia cell line). Cells were fixed with 2% formaldehyde for 10 minutes at room temperature. ab13556 was used at 2 µg/10^6 cells for 60 minutes at 37°C (green). Goat Anti- Rabbit Dylight 488 was used as a secondary antibody at 1/200 dilution for 40 minutes at 37°C. Isotype control was Rabbit IgG under the same conditions (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TLR4 antibody (ab13556)

TLR4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse spleen tissue using rabbit Anti-TLR4 antibody

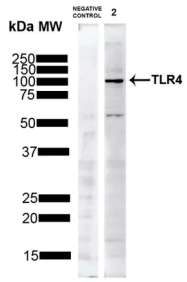
Immunohistochemistry analysis using Anti-TLR4 antibody (ab13556). Positive staining on mouse spleen tissue. Primary antibody ab13556 was used at 1:100.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TLR4 antibody (ab13556)

TLR4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse colon colitis (Localization: Inflammatory cells) using rabbit Anti-TLR4 antibody

Immunohistochemistry analysis using Anti-TLR4 antibody. Positive staining on mouse colon colitis. Primary antibody was used at a dilution of 1:100000 for 12 hours at 4°C. Secondary Antibody was biotin Goat Anti-Rabbit at 1:2000 for 1 hour at RT. Counterstain: Methyl Green at 200uL for 2 min at RT. Localization: Inflammatory cells.



Western blot - Anti-TLR4 antibody (ab13556)

TLR4 Western blot staining of HeLa cell lysate using rabbit Anti-TLR4 antibody

Blocking buffer: 5% Skim Milk powder in TBST.
Lane 1:
No primary antibody

Lane 2:
Western blot - Anti-TLR4 antibody (ab13556) at 1/1000 dilution

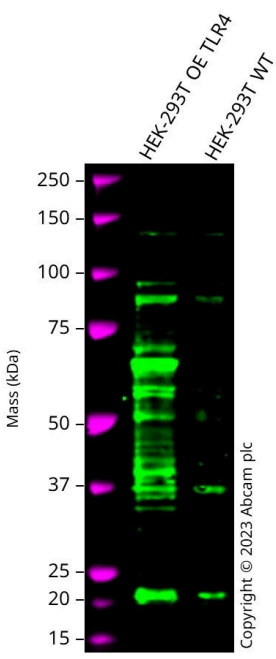
All lanes:
HeLa cell lysate at 10 µg

Secondary

All lanes:
HRP Goat Anti-Rabbit IgG at 1/4000 dilution

Predicted band size: 75 kDa

Observed band size: 75 kDa



Western blot - Anti-TLR4 antibody (ab13556)

Western blot: Anti-TLR4 antibody (ab13556) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab13556 was shown to bind specifically to TLR4. First, unboiled samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-TLR4 antibody (ab13556) at 1/1000 dilution

Lane 1:
HEK-293T overexpressing TLR4 (unboiled) cell lysate at 20 µg

Lane 2:
HEK-293T untransfected (unboiled) cell lysate at 20 µg

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 70 kDa

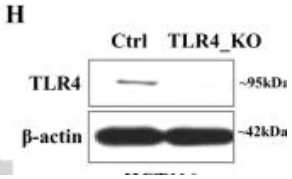


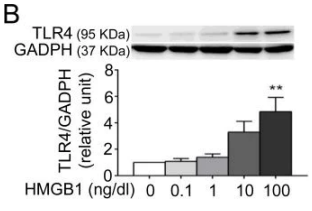
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Western blot - Anti-TLR4 antibody (ab13556)

TLR4 Western blot staining using rabbit Anti-TLR4 antibody

TLR4 western blotting using Anti-TLR4 antibody ab13556. Publication image and figure legend from Hu, X., Fatima, S., et al., 2021, Cell Death Dis, Pubmed 34385421.

Palmitic acid increases CRC cell proliferation in a TLR4-dependent manner.Protein expression of TLR4, MyD88, and TIRAP in A SW480 and B HCT116 cells after palmitic acid (PA, 50 µm) treatments. C Kinetics analysis of palmitic acid binding to TLR4 protein based on SPR platform Biacore X100. Representative sensorgrams were obtained from injections of palmitic acid at different concentrations as indicated. Expression of p-NFκB in D SW480 and E HCT116 cells after palmitic acid (PA, 50 µm) treatments. Proliferation of F SW480 and G HCT116 cells after palmitic acid (PA, 50 µm) treatments in the presence or absence of C34. H Knockout of TLR4 in HCT116 cells (HCT116TLR4-KO). I Proliferation of HCT116 cells and HCT116TLR4-KO cells after palmitic acid (PA, 50 µm) treatments in the presence or absence of C34. Data are shown as means ± SEM. n = 3 independent experiments. *p < < 0.05, **p < < 0.01 as indicated. Fatty acid treatments were done in the presence of 1% fatty acid-free BSA, 1% fatty acid-free BSA alone served as control (Ctrl).



Western blot - Anti-TLR4 antibody (ab13556)

TLR4 Western blot staining using rabbit Anti-TLR4 antibody

TLR4 western blotting using Anti-TLR4 antibody ab13556. Publication image and figure legend from Lai, C. H., Wang, K. C., et al., 2016, PLoS One, Pubmed 26741694.

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TLR4 mediates cytokine production in HASMCs.(A) Basal levels of IL-6 and MCP-1 were assessed after being cultured in fresh medium without stimulation for 24 hours (n = 4 per group). (B, C, D) The protein expression of TLR4 in cell lysates (B) and levels of IL-6 (C) and MCP-1 (D) in supernatants were assessed after HMGB1 treatment for 24 hours (n = 4 per group). (E) Levels of IL-6 and MCP-1 were assessed after HMGB1 treatment (100 ng/dl) for 24 hours (n = 4 per group). (*P<0.05, **P<0.01 compared with untreated or control siRNA group.)

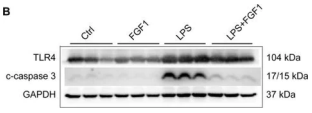


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Western blot - Anti-TLR4 antibody (ab13556)

TLR4 Western blot staining using rabbit Anti-TLR4 antibody

TLR4 western blotting using Anti-TLR4 antibody ab13556. Publication image and figure legend from Dhlamini, Q., Wang, W., et al., 2022, Mol Med, Pubmed 35764933.

The effects of FGF1 pretreatment of LPS-induced cell death. A Representative images of TUNEL assay staining to detect apoptotic cells in the lung sections of mice at 12- and 24 h. Scale bar = 100 μm. Apoptotic cells were observed under fluorescence microscopy and the percentage of TUNEL positive cells per 40x magnification field was calculated. B WB analysis and quantification of TLR4 and the downstream apoptosis-related protein cleaved-caspase 3 in murine lungs. WB assays were performed at 12 h post LPS treatment. Data are presented as mean ± SD (n = 3 per group). **p < < 0.01, ***p < < 0.001, ****p < < 0.0001; One-way ANOVA with Tukey's post-hoc test

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