**Product datasheet**

**Anti-TLR4 antibody [76B357.1] ab22048**

**Overview**

**Product name**  
Anti-TLR4 antibody [76B357.1]

**Description**  
Mouse monoclonal [76B357.1] to TLR4

**Host species**  
Mouse

**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.

**Tested applications**  
Suitable for: ELISA, IHC-P, IHC-Fr, Flow Cyt, ICC, ICC/IF

**Species reactivity**  
Reacts with: Mouse, Rat, Human, Pig

**Predicted to work with:** Sheep, Horse, Cow, Cat, Chimpanzee

**Immunogen**  
Synthetic peptide corresponding to Human TLR4 aa 100-200 conjugated to Keyhole Limpet Haemocyanin (KLH).

**Database link:** O00206

**Positive control**  
Human, Mouse and Rat small intestine for Western Blot or THP1 cells for FACS analysis. Jurkat cells.

**Properties**

**Form**  
Liquid

**Storage instructions**  

**Storage buffer**  
Preservative: 0.05% Sodium azide  
 Constituents: 99% PBS, 0.05% BSA

**Purity**  
Protein G purified

**Clonality**  
Monoclonal

**Clone number**  
76B357.1

**Isotype**  
IgG2b

**Light chain type**  
kappa
Function

Cooperates with LY96 and CD14 to mediate the innate immune response to bacterial lipopolysaccharide (LPS). Acts via MYD88, TIRAP and TRAF6, leading to NF-κappa-B activation, cytokine secretion and the inflammatory response. Also involved in LPS-independent inflammatory responses triggered by Ni(2+). These responses require non-conserved histidines and are, therefore, species-specific.

Tissue specificity

Highly expressed in placenta, spleen and peripheral blood leukocytes. Detected in monocytes, macrophages, dendritic cells and several types of T-cells.

Involvement in disease

Genetic variation in TLR4 is associated with age-related macular degeneration type 10 (ARMD10) [MIM:611488]. ARMD is a multifactorial eye disease and the most common cause of reversible vision loss in the developed world. In most patients, the disease is manifest as ophthalmoscopically visible yellowish accumulations of protein and lipid that lie beneath the retinal pigment epithelium and within an elastin-containing structure known as Bruch membrane.

Sequence similarities

Belongs to the Toll-like receptor family.
Contains 18 LRR (leucine-rich) repeats.
Contains 1 LRRCT domain.
Contains 1 TIR domain.

Domain

The TIR domain mediates interaction with NOX4.

Post-translational modifications

N-glycosylated. Glycosylation of Asn-526 and Asn-575 seems to be necessary for the expression of TLR4 on the cell surface and the LPS-response. Likewise, mutants lacking two or more of the other N-glycosylation sites were deficient in interaction with LPS.

Cellular localization

Membrane.

Applications

Our Abpromise guarantee covers the use of ab22048 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 24952384</td>
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<tr>
<td>IHC-P</td>
<td>5/5</td>
<td>1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Antigen retrieval with citrate buffer pH 6.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>5/5</td>
<td>1/100.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>3/5</td>
<td>Use 1μg for 10^6 cells. Methanol or paraformaldehyde fixed cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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</table>
Representative examples of immunohistochemical staining of TLR4 in colorectal carcinoma tissues (original magnification 100×).

Paraffin-embedded human normal colorectal tissue (A) and colorectal carcinoma tissue (D) stained for TLR4 with ab22048 in immunohistochemical analysis. Counter stained with hematoxylin. No staining is observed in normal colorectal tissue (A).

(From Figure 4A and 4D of Xu et al)

ab22048 staining TLR4 in Human stomach tissue sections by Immunohistochemistry (IHC-P - formaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 5% serum for 1 hour; antigen retrieval was by heat mediation in citrate buffer (10mM, pH 6) (ab64236). Samples were incubated with primary antibody (1/100) for 1 hour at 23°C. An undiluted HRP-conjugated goat anti-mouse IgG polyclonal was used as the secondary antibody.

Overlay histogram showing Jurkat cells (ab7899) stained with ab22048 (red line). The cells were fixed with methanol (5 min) and incubated in 1x PBS / 10% normal goat serum (ab7481) / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab22048, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with 4% paraformaldehyde (10 min) used under the same conditions.

Please note that Abcam do not have data for use of this antibody on non-fixed cells. We welcome any customer feedback.
Immunohistochemistry (Frozen sections) - Anti-TLR4 antibody [76B357.1] (ab22048)

This image is courtesy of an Abreview submitted by Laura McCulloch

ab22048 staining TLR4 in Mouse spleen tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with acetone and blocked with 10% serum for 30 minutes at 20°C. Samples were incubated with primary antibody (1/100 in PBS-BSA) for 1 hour at 20°C. An Alexa Fluor®488-conjugated Rat anti mouse IgG polyclonal (1/200) was used as the secondary antibody.

TLR4 labelling (yellow) co-localises with CD35 labelling (red) on follicular dendritic cells in the spleen. TLR 4 alone (green) can also be seen on other cell types in the spleen- most probably macrophages.

Immunocytochemistry/ Immunofluorescence - Anti-TLR4 antibody [76B357.1] (ab22048)

This image is courtesy of an Abreview submitted by Laura McCulloch

Immunofluorescence analysis of murine macrophages, staining TLR4 with ab22048.

Cells were fixed with formaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 1% BSA for 10 minutes at 25°C. Samples were incubated with primary antibody (1/100 in 1% BSA) for 12 hours at 4°C. A TexasRed®-conjugated donkey anti-mouse (ab6818) polyclonal IgG (1/100) was used as the secondary antibody.
ab22048 (5 µg/ml) staining TLR4 in Human skin tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Membrane-cytoplasmic immunopositivity of TLR4 was primarily observed in the pigmented basal cells and the adjacent keratinocytes in the epidermal layer.

ab22048 at 5 µg/ml staining TLR4 in Human colon tissue sections by Immunohistochemistry (Formalin/ PFA-fixed paraffin-embedded tissue sections). Left hand image shows staining with an isotype control antibody whilst right one shows staining with ab22048.

ab22048 staining TLR4 in Rat's salivary gland tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). ab22048 at a dilution of 1:100 generated a membrane-cytoplasmic staining in the tissue with stronger signal in ductal epithelial cells.

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