## abcam

#### Product datasheet

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

★★★★★ 28 Abreviews 261 References 7 Images

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: Flow Cyt (Intra), ICC/IF, IHC-P, ICC, IHC-Fr, ELISA

**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: **000206** 

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

Cyt (Intra): Jurkat cells.

General notes

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

**Properties** 

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

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Storage buffer pH: 7.40

Preservative: 0.05% Sodium azide Constituents: 99% PBS, 0.05% BSA

Purity Protein G purified

ClonalityMonoclonalClone number76B357.1IsotypeIgG2bLight chain typekappa

#### **Applications**

The Abpromise guarantee

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.

Application	Abreviews	Notes	
Flow Cyt (Intra)		Use 1µg for 10 <sup>6</sup> cells.  Methanol or paraformaldehyde fixed cells. <b>ab170192</b> - Mouse monoclonal lgG2b, is suitable for use as an isotype control with this antibody.	
ICC/IF	<b>★★★★★</b> (5)	Use at an assay dependent concentration.	
IHC-P	<b>★★★★★</b> (5)	Use at an assay dependent concentration.	
ICC	<b>★★★★</b> <u>(3)</u>	Use at an assay dependent concentration.	
IHC-Fr	<b>★★★★★</b> (2)	Use at an assay dependent concentration.	
ELISA		Use at an assay dependent concentration. PubMed: 24952384	

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**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-kappa-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

irreversible 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

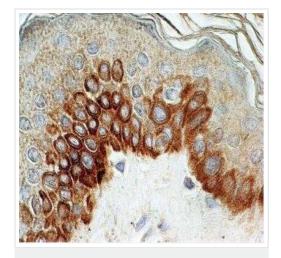
N-glycosylated. Glycosylation of Asn-526 and Asn-575 seems to be necessary for the expression of TLR4 on the cell surface and the LPS-response. Likewise, mutants lacking two or more of the

other N-glycosylation sites were deficient in interaction with LPS.

**Cellular localization** 

Membrane.

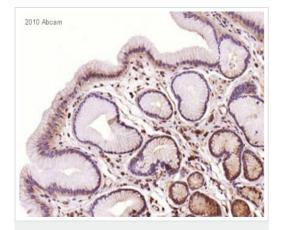
#### **Images**



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TLR4 antibody
[76B357.1] (ab22048)

Immunohistochemical analysis of paraffin-embedded Human skin tissue labeling TLR4 with ab22048 at 5 ug/ml. The section was blocked using blocking solution (1% BSA in PBS) for 1 hour at room temperature and incubated with ab22048 overnight at 4°C after removing blocking solution.

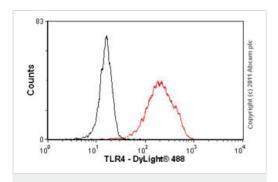
Antigen retrieval: Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TLR4 antibody [76B357.1] (ab22048)

This image is courtesy of an anonymous Abreview

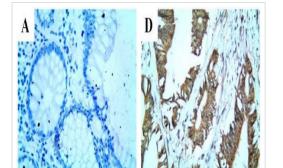
ab22048 staining TLR4 in Human stomach tissue sections by Immunohistochemistry (IHC-P - formaldehyde-fixed, paraffinembedded sections). Tissue was fixed with formaldehyde and blocked with 5% serum for 1 hour; antigen retrieval was by heat mediation in citrate buffer (10mM, pH 6) (ab64236). Samples were 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.



Flow Cytometry (Intracellular) - Anti-TLR4 antibody [76B357.1] (ab22048)

Overlay histogram showing Jurkat cells (<u>ab7899</u>) stained with ab22048 (red line). The cells were fixed with methanol (5 min) and incubated in 1x PBS / 10% normal goat serum (<u>ab7481</u>) / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab22048,  $1\mu g/1x10^6$  cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse lgG (H+L) (<u>ab96879</u>) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse lgG2b [PLPV219] (<u>ab91366</u>,  $2\mu g/1x10^6$  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 (Formalin/PFA-fixed paraffinembedded sections) - Anti-TLR4 antibody [76B357.1] (ab22048)

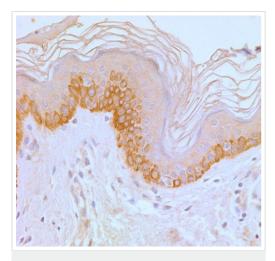
Xu H. et al PLoS One. 2011;6(12):e27399. doi: 10.1371/journal.pone.0027399. Epub 2011 Dec 13 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

# 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.

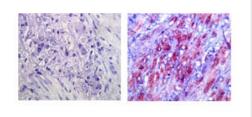
No staining is observed in normal colorectal tissue (A).

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



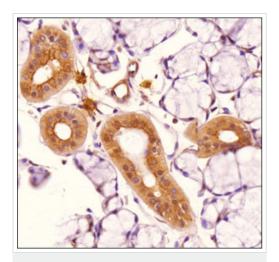
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TLR4 antibody [76B357.1] (ab22048)

ab22048 (5 ug/ml) staining TLR4 in Human skin tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Membrane-cytoplasmic immunopositivity of TLR4 was primarily observed in the pigmented basel cells and the adjacent keratinocytes in the epidermal layer.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TLR4 antibody [76B357.1] (ab22048)

ab22048 at 5  $\mu$ g/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.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TLR4 antibody [76B357.1] (ab22048)

ab22048 staining TLR4 in Rat's salivary gland tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded 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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