

## Product Information

Contents: Affinity Purified anti-human Toll-like receptor 9 (CD289, TLR9, TLR-9)

Catalog Number: 14-9099

Sizes: 25 ug, 100 ug

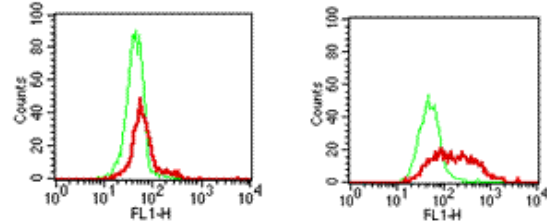
Formulation: Phosphate buffer pH 7.2, 150 mM NaCl, 0.09% NaN<sub>3</sub>

Storage Conditions: Store at 4°C.

Avoid repeated freeze/thaw cycles.

Clone: eB72-1665

Isotype: Rat IgG2a, κ



HEK 293 parent cells (left panel) and 293/hTLR9 transfected cells (right panel) were fixed and permeabilized and subsequently stained with either anti-rat IgG-FITC (cat. 11-4811) alone (thin line) or with 1 µg of eB72-1665 followed by anti-rat IgG-FITC (thick line).

### Available Formats of This Product

Cat. No.	Format	Excite (nm)	Emit (nm)	Reported Applications
12-9099	PE anti-human Toll-like receptor 9 (CD289, TLR9, TLR-9)	488	575	IC Flow
14-9099	Affinity Purified anti-human Toll-like receptor 9 (CD289, TLR9, TLR-9)	N/A	N/A	IC Flow IP WB

Questions? Please consult our answers to frequently asked questions at <http://www.ebioscience.com/faq>.

### Description

eB72-1665 is generated against a portion of human toll-like receptor 9 (aa 273-288), a molecule reported to be expressed predominantly intracellularly. TLR9 is a ~115-120 kDa molecule, which mediates response to unmethylated CpG dinucleotides in bacterial DNA. CpG DNA induces a strong T-helper-1-like inflammatory response and the proliferation of TLR9-positive human B cells. When stimulated with CpG DNA, TLR9-deficient (TLR9<sup>-/-</sup>) mice lacked splenocyte proliferation, inflammatory cytokine production from macrophages, and dendritic cell maturation, as compared with normal mice. To date, at least twelve members of the Toll family have been identified. This family of type I transmembrane proteins is characterized by an extracellular domain with leucine-rich repeats and a cytoplasmic domain with homology to the type I IL-1 receptor. Members of the TLR family are involved in recognition and response to different microbial components including lipoproteins, peptidoglycans, and nucleic acids and play important roles in innate immunity and inflammation. TLR9 is not detected by flow cytometry using this antibody on lysed whole human blood and/or isolated human PBMC stained for cell surface or intracellular TLR9. This may be due to limitations of antigen detection by flow cytometry. Human pDCs matured in the presence of IL-3 have been reported to stain with eB72-1665 by immunofluorescence microscopy (Nat Immunol. 5:190). Human Epithelial Cell lines were also reported to stain with this mAb (J. Immunol. 173: 1219). Further studies are needed to determine the relationship between mRNA expression and protein detection by flow cytometry.

### Applications Reported

For research use only, not for diagnostic or therapeutic use. This eB72-1665 antibody has been reported for use in intracellular flow cytometric analysis, immunoprecipitation, and immunoblotting (WB). (Fluorochrome conjugated eB72-1665 is recommended for use in intracellular flow cytometry.) Preliminary in vitro blocking assays suggested that eB72-1665 may not be effective in blocking of CpG binding. Please contact us for further information.

### Applications Tested

The eB72-1665 antibody has been tested by intracellular flow cytometric analysis of hTLR9 transfected cells. This can be used at less than or equal to 1 µg per million cells in a 100 µl total staining volume. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

### References

Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, Lien E, Nilsen NJ, Espevik T, Golenbock DT. 2004. TLR9 signals

after translocating from the ER to CpG DNA in the lysosome. *Nat Immunol.* 5(2):190-8.

Juliane Platz, Christoph Beisswenger, Alexander Dalpke, Rembert Koczulla, Olaf Pinkenburg, Claus Vogelmeier, and Robert Bals. 2004. Microbial DNA Induces a Host Defense Reaction of Human Respiratory Epithelial Cells. *J. Immunol.* 173: 1219 - 1223.

Leifer, CA, Kennedy, MN, Mazzone, A, Lee, CW, Kruhlak, MJ, Segal, DM. 2004. TLR9 is Localized in the Endoplasmic Reticulum Prior to Stimulation. *J Immun.* 173:1179-1183.

Jozsef L, Khreiss T, El Kebir D, Filep JG. Activation of TLR-9 induces IL-8 secretion through peroxynitrite signaling in human neutrophils. *J Immunol.* 2006 Jan 15;176(2):1195-202. (eB72-1665, FC, PubMed)

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#### Related Products

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- Cat. 14-4321 Affinity Purified Rat IgG2a Isotype Control
- Cat. 14-8185 Recombinant B18R protein (Vaccinia Virus-Encoded Neutralizing Type I Interferon Receptor; Type I IFN inhibitor)
- Cat. 34-8185 Carrier-Free Recombinant B18R protein (Vaccinia Virus-Encoded Neutralizing Type I Interferon Receptor; Type I IFN inhibitor)
- Cat. 00-8222 eBioscience IC Fixation Buffer
- Cat. 11-8317 Fluorescein isothiocyanate (FITC) mouse anti-human IFN-alpha2 (clone 225.C)
- Cat. 12-8317 Phycoerythrin (PE) mouse anti-human IFN-alpha2 (clone 225.C)
- Cat. 17-8317 Allophycocyanin (APC) mouse anti-human IFN-alpha2 (clone 225.C)
- Cat. 00-8333 eBioscience Permeabilization Buffer(10X)
- Cat. 88-8823 eBioscience Fixation & Permeabilization
- Cat. 66-P929 Toll-like receptor 9 (TLR9, TLR-9) blocking peptide (for 14-9929-92)

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