

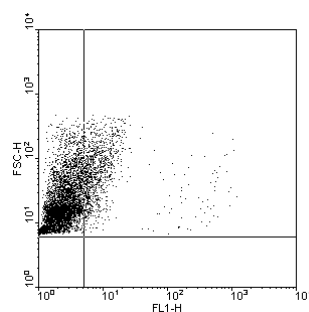
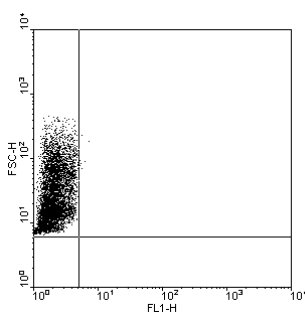
## CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

<b>Product name</b>	TLR9, Mouse, clone 5G5, biotinylated		
<b>Catalog number</b>	HM1042BT-50UG		
<b>Lot number</b>	-	<b>Expiry date</b>	-
<b>Volume</b>	500 µl	<b>Amount</b>	100 µg
<b>Formulation</b>	0.2 µm filtered in PBS+0.1%BSA+0.02%NaN3	<b>Concentration</b>	100 µg/ml
<b>Host Species</b>	Mouse IgG2a	<b>Conjugate</b>	None
<b>Endotoxin</b>	N.A.	<b>Purification</b>	Protein G
<b>Storage</b>	4°C		

### Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #	7	3	1,3	1,5,6,8				1-4
Yes	•	•	•	•				•
No								
N.D.					•	•	•	

N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot



FC: THP1 cells were incubated with IgG2a isotype controle. Cells (140000) were permeabilized with saponin and stained with 0.4 µg 5G5.

FC: THP1 cells were incubated with α-TLR9 5G5 mAb. Cells (140000) were permeabilized with saponin and stained with 0.4 µg 5G5.

Dilutions to be used depend on detection system applied. It is recommended that users test the reagent and determine their own optimal dilutions. The typical starting working dilution is 1:50.

- F: 10µm sections were fixed with acetone for 10 minutes. PBS washed sections were incubated with 5G5 1:100 in 1% BSA for 30 minutes at RT. (Ref.7)
- IF: cells were fixed with 2% formalin for 15 minutes at RT and permeabilized with a mAb (4µg/400µl) containing buffer (PBS, 0.2% BSA, 0.2% saponin) for 1 hour. (Ref.1)
- FC: RAW264.7 cells were fixed for 15 minutes with 4% formalin and permeabilized (PBS, 0.5%BSA, 0.5% saponin) at RT. (Ref.1)
- P: paraffin embedded tissues 5µm sections were made. After antigen retrieval (0.01mol/l, pH6 sodium citrate) and quenching of endogenous peroxidase, sections were blocked with 0.5% ovalbumin and 0.1% gelatin for 20 minutes at RT. Sections were incubated with 5G5 for 1 hour at 37°C. (Ref.3)
- W: reduced lysates were resolved by 10% SDS-PAGE and blotted on nitrocellulose. After blocking with 5% skimmed milk TLR9 was detected with 2µg/ml 5G5. (Ref.1)
- Positive control: RAW264.7 macrophages stimulated with IFN $\gamma$ .

### General Information

#### Description

The monoclonal antibody 5G5 reacts with the Toll-like receptor 9 (TLR9, CD289). TLRs are highly conserved throughout evolution and have been implicated in the innate defence to many pathogens. In Drosophila, toll is required for the anti-fungal response, while the related 18-wheeler is involved in antibacterial defences. In mammals, TLRs identified as type I transmembrane signalling receptors with pattern recognition capabilities, have been implicated in the innate host defence to pathogens. As investigated so far all functional characterized TLR signal via the TLR/IL-1 receptor (IL-1R) pathway where recruitment of MyD88 seems to be essential. In contrast to cell-wall components, bacterial DNA is probably invisible for immune cells until DNA is liberated during processes taking place in the

endosomal/lysosomal compartment where intracellular TLR9 recruits MyD88 to initiate signal transduction. Unmethylated CpG-dinucleotide-containing sequences are found much more frequently in bacterial genomes than in vertebrates genomes, whereas the frequency of CpG dinucleotides are suppressed and usually methylated. The regions adjacent to the CpG dinucleotides also affect the immunostimulatory activity. The optimal sequence differs significantly between mammalian species. Methylated CpG dinucleotides lack immunostimulatory activities. Cellular activation in response to bacterial DNA and synthetic dinucleotides containing unmethylated CpG-dinucleotides is mediated by TLR9. The monoclonal antibody 5G5 reacts with RAW macrophages and TLR9 transfected HEK293 cells, and it is cross reactive with human and canine TLR9.

<b>Immunogen</b>	Purified fusion protein of extracellular domain of human TLR9 (AA 1-815) and human IgGFc
<b>Aliases</b>	CD289, TLR9, Toll-like receptor 9.
<b>Cross reactivity</b>	Canine: Yes; Human: Yes
<b>References</b>	<ol style="list-style-type: none"><li>1. Ahmad-Nejad, P et al; Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. <i>Eur J Immunol</i> 2002, <i>32</i>: 1958</li><li>2. Rutz, M et al; Toll-like receptor 9 binds single-stranded CpG-DNA in a sequence- and pH-dependent manner. <i>Eur J Immunol</i> 2004, <i>34</i>: 2541</li><li>3. Rumio C et al; Degranulation of Paneth cells via Toll-like receptor 9. <i>Am J Pathol</i> 2004, <i>165</i>:373</li><li>4. Pratesi, G et al; Therapeutic synergism of gemcitabine and CpG-oligodeoxynucleotides in an orthotopic human pancreatic carcinoma xenograft. <i>Cancer res</i> 2005, <i>65</i>: 6388</li><li>5. Tokumasa, N et al; Expression of Tyk2 in dendritic cells is required for Il-2, Il-23, and IFN<math>\gamma</math> production and the induction of Th1 cell differentiation. <i>Blood</i> 2007, <i>110</i>: 553</li><li>6. Burgener, I et al; Antibodies specific for human or murine Toll-like receptors detect canine leukocytes by flow cytometry. <i>Vet Immunol Immunopathol</i> 2008, <i>124</i>: 184</li><li>7. Machida, H et al; Expression of Toll-like receptor 9 in renal podocytes in childhood-onset active and inactive lupus nephritis. <i>Nephrol Dial Transplant</i> 2010, <i>25</i>: 2530</li><li>8. Zheng, W et al. Distinct host-related dendritic cell responses during the early stage of <i>Plasmodium yoelii</i> infection in susceptible and resistant mice. <i>Parasite immunology</i> 2010, <i>32</i>: 324</li></ol>
<b>Storage&amp;stability</b>	Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.
<b>Precautions</b>	For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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We hereby certify that the above-stated information is correct and that this product has been successfully tested by the Quality Control Department. This product was released for sale according to the existing specifications. This document has been produced electronically and is valid without a signature.

Approved by Manager of QC  
Brenda Teunissen

Date  
04/11/2019

Do you have any questions or comments regarding this product? Please contact us via [support@hycultbiotech.com](mailto:support@hycultbiotech.com).