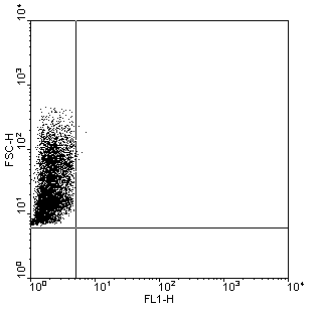
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	TLR9, Mouse, clone 5G5, FITC conjugated		
Catalog number	HM1042F-20UG		
Lot number	-	**Expiry date**	-
Volume	200 µl	**Amount**	20 µg
Formulation	0.2 µm filtered in PBS+1%BSA+0.02%NaN3	**Concentration**	100 µg/ml
Host Species	Mouse IgG2a	**Conjugate**	FITC
Endotoxin	N.A.	**Purification**	Protein G
Storage	4°C		

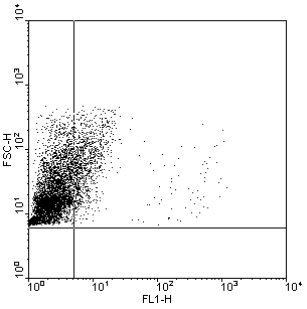
Application notes

	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 isptype 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 IFNγ.

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.

Immunogen Purified fusion protein of extracellular domain of human TLR9 (AA 1-815) and human IgGFc

Aliases CD289, TLR9, Toll-like receptor 9.

Cross reactivity Canine: Yes; Human: Yes

References

1. Ahmad-Nejad, P et al; Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. Eur J Immunol 2002, *32*: 1958
2. Rutz, M et al; Toll-like receptor 9 binds single-stranded CpG-DNA in a sequence- and pH-dependent manner. Eur J Immunol 2004, *34*: 2541
3. Rumio C et al; Degranulation of Paneth cells via Toll-like receptor 9. Am J Pathol 2004, *165*:373
4. Pratesi, G et al; Therapeutic synergism of gemcitabine and CpG-oligodeoxynucleotides in an orthotopic human pancreatic carcinoma xenograft. Cancer res 2005, *65*: 6388
5. Tokumasa, N et al; Expression of Tyk2 in dendritic cells is required for Il-2, Il-23, and IFNγ production and the induction of Th1 cell differentiation. Blood 2007, *110*: 553
6. Burgener, I et al; Antibodies specific for human or murine Toll-like receptors detect canine leukocytes by flow cytometry. Vet Immunol Immunopathol 2008, *124*; 184
7. Machida, H et al; Expression of Toll-like receptor 9 in renal podocytes in childhood-onset active and inactive lupus nephritis. Nephrol Dial Transplant 2010, *25*: 2530
8. Zheng, W et al. Distinct host-related dendritic cell responses during the early stage of *Plasmodium yoelii* infection in susceptible and resistant mice. Parasite immunology 2010, *32*: 324

Storage&stability Product should be stored at 4°C. Under recommended storage conditions, product is stable for at least one year.

Precautions 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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Brenda Teunissen	13/07/2021

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