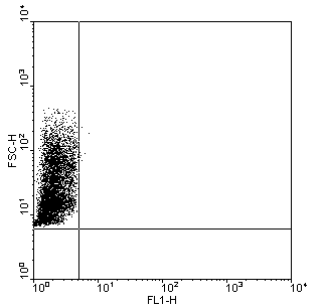
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

Product name	TLR9, Human, clone 5G5, FITC conjugated		
Catalog number	HM2087F-100UG		
Lot number	-	**Expiry date**	-
Volume	1 ml	**Amount**	100 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		

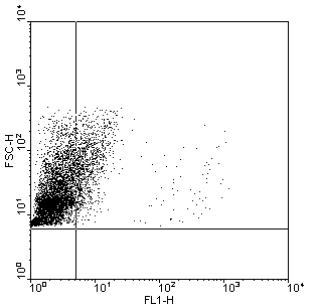
Application notes

	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 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 IFNγ.

General Information

Description

The monoclonal antibody 5G5 recognizes human Toll-like receptor 9. Toll-like receptors (TLRs) are highly conserved from Drosophila to humans and share structural and functional similarities. TLRs constitute of a family of pattern recognition receptors (PRRs) that mediate cellular responses to a large variety of pathogens (viruses, bacteria, and parasites) by specific recognition of so-called 'pathogen-associated molecular patterns'. Activation of TLRs, a family of at least 11 different members that function either as homo- or heterodimers, leads to activation of NFκB-dependent and IFN-regulatory factor-dependent signaling pathways. TLRs have a central role in innate immunity and are also required for the development of an adaptive immune response. TLRs are expressed by various cells of the immune

system, such as macrophages and dendritic cells. They recognize and respond to molecules derived from bacterial, viral and fungal pathogens. Whereas most TLRs are expressed on the cell surface, TLR9 is expressed intracellularly within one or more endosomal compartments and recognizes nucleic acids. TLR9 detects a rather subtle difference in the DNA of vertebrates compared with that of pathogens. Vertebrate genomic DNAs have mostly methylated CpG dinucleotides where bacterial and viral DNAs have unmethylated CpG dinucleotides. TLR9 undergoes relocation from endoplasmic reticulum to CpG-ODN-containing endosomes. In these endosomes TLR9 becomes a functional receptor after proteolytic cleavage. TLR9 exists as a preformed homodimer and CpG-ODN binding promotes its conformational change, bringing the cytoplasmic TIR-like domains close to each other. This allows a recruitment of the key adapter protein MyD88 which initiates a signalling cascade. The only human immune cell types known to constitutively express TLR9 and to be activated by CpG ODN are pDCs and B cells. TLR9 triggering induces an activation phenotype in the B cells and pDCs, characterized by the expression of costimulatory molecules, resistance to apoptosis, and induces Th1-type immune response profiles.

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; Mouse: 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.

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	Date
Brenda Teunissen	02/12/2019

Do you have any questions or comments regarding this product? Please contact us via support@hycultbiotech.com.