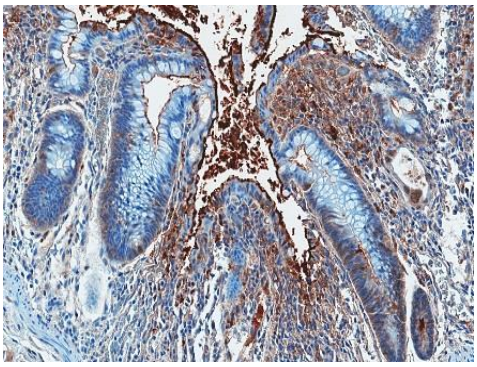
CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

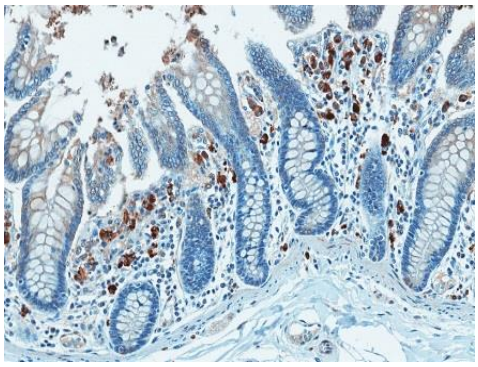
Product name	Lipopolysaccharide Core, clone WN1 222-5		
Catalog number	HM6011-1MG		
Lot number	xxxxxXxxxx-X	**Expiry date**	MMM YYYY
Volume	xx ml	**Amount**	1 mg
Formulation	0.2 µm filtered in PBS	**Concentration**	>0.5 mg/ml
Host Species	Mouse IgG2a	**Conjugate**	None
Endotoxin	<24 EU/mg	**Purification**	Protein G
Storage	4°C		

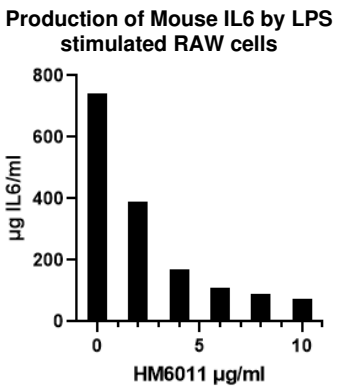
Application notes

	IHC-F	IHC-P	IF	FC	FS	IA	IP	W
Reference #		6				1,4		1,5
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







IHC-P: IHC-P: analysis of lipopolysaccharide core in paraffin-embedded rhesus macaques colon tissue using mAb WN1 222-5 (Cat.# HM6011). Images are kindly provided by Dr. Jacob D. Estes, AIDS & Cancer Virus Program, SAIC-Frederick Inc., Frederick National Laboratory for Cancer Research.

FS: RAW cells were incubated with LPS and HM6011. The supernatant was measured using mouse IL6 ELISA.

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.

- WB: reduced and non-reduced MC/9 lysate; band size 38kDa under reducing conditions and 74 kDa under nonreducing conditions
- W: A non-reduced sample treatment and SDS-PAGE was used (Ref. 1 and 5).
- IHC-P: Antigen retrieval was performed by heating sections in 1x DIVA Decloaker reagent.. As positive control gut or lymph node of SIV-infected rhesus macaques was used and as negative control gut or lymph node of non-SIV-infected rhesus macaques (Ref.6).
- Positive control: LPS *E.coli*; Negative control: Normal Lymph node (non-infected)
- FS: RAW cells were seeded in a 48 well plate at 10x103 RAW cells/well. Plate was pre-incubated for 4 hours at 370C/5% CO2. An LPS stock of 2.5 µg/ml was made and different concentrations of HM6011 antibody was added and pre-incubated for 20 min at 40C. After pre-incubation the LPS/HM6011 mix was added to the RAW cells. Culture was incubated for 16 hours at 370C/5% CO2. Supernatants were harvested. Mouse IL6 levels were measured using mouse IL6 ELISA (R&D systems; cat.no. DY406-05).

General Information

Description

The mouse monoclonal antibody clone WN1 222-5 recognizes the core region of lipopolysaccharide (LPS), whereas it lacks reactivity with free lipid A and Rd2 or smaller LPS. LPS is a constituent of the outer membrane of the cell wall of certain types of Gram-negative bacteria, such as E. coli, Salmonella, Shigella, Pseudomonas, Neisseria, Haemophilus, and some other lesser known pathogens. The endotoxins of Gram-negative bacteria are LPS molecules with three distinct domains referred to as lipid A, core oligosaccharide and O-polysaccharide. The lipid A and core oligosaccharide

comprise the endotoxin core and are relatively conserved among different Gram-negative bacterial species. The O-polysaccharides show wide structural diversity and give rise to the O-specificity of different strain and species serotypes. Lipid A is the toxic moiety of endotoxin, and is covalently linked to core oligosaccharide in all LPS. Rough (R) bacteria lack the O-polysaccharide of their smooth (S) strain bacteria counterparts, and different rough mutant bacteria have been isolated expressing a range of incomplete core oligosaccharide structures ranging from complete core (Ra) to deep-rough (Re) mutants expressing only lipid A linked to inner-core KDO residues.
During life, humans as well as other vertebrates, are often exposed to LPS, for instance by enterobacteria. While growing, enterobacteria releases small amounts of endotoxins. Predominant part of the endotoxins stay on the cell wall until the bacterium disintegrates. Endotoxins are heat stable, so even boiling the infected material for 30 minutes will not denature it. Watery diarrhea is caused by released LPS that interacts with the digestive track intestine. Cells and tissue can be destroyed by the LPS resulting in inflammation. Furthermore, LPS is a causal factor of NEC (necrotizing enterocolitis), a disorder found mostly in newborn infants. If LPS gains entry to the bloodstream, it can bind the host cells, such as macrophages. LPS responses are complicated and involve complexes of LPS-binding protein (LBP), soluble and/or membrane bound CD14 and Toll like receptor 4 (TLR4). LBP is a serum protein that catalyzes LPS recognition by CD14. Recognition of LPS triggers a cascade of adverse systemic responses and organ failure (septic shock).

Immunogen 10^8 heat-killed bacteria

Aliases LPS core

Cross reactivity Core region of LPS, S-form: Yes; Core region of LPS, R-form: Yes

References

1. Di Padova, FE et al; Identification of widely cross-reactive and cross-protective anti-LPS core monoclonal antibodies (Mabs). Infect Immun 1993, *61*:3863
2. Bahrami, S et al; Monoclonal antibody to endotoxin attenuates hemorrhage-induced lung injury and mortality in rats. Crit Care Med 1997, *25*: 1030
3. Pollack, M et al; Dual effects of lipopolysaccharide (LPS) antibodies on cellular uptake of LPS and LPS-induced proinflammatory functions. J Immunol 1997, *151*: 3519
4. Müller-Loennies, S et al; Identification of a cross-reactive epitope widely present in lipopolysaccharide from enterobacteria and recognized by the cross protective monoclonal antibody WN1 222-5. J Biol Chem 2003, *278*: 25618
5. Tsuneyoshi, N et al; The functional and structural properties of MD-2 required for lipopolysaccharide binding are absent in MD-1. J Immunol 2005, *174*: 340
6. Estes, J et al; Damaged intestinal epithelial integrity linked to microbial translocation in pathogenic simian immunodeficiency virus infections. PLOS pathogens 2010, *6*: e1001052
7. Wang, A et al; Anti-LPS Test Strip for the Detection of Food Contaminated with Salmonella and E. coli. Microbial Biochem Technol 2011, *3*:2
8. Barclay, G et al; Monoclonal Antibodies to Endotoxin Core Segregate into Families of Specificity and Cross-Reactivity to Lipopolysaccharides in ELISA by Correlation Cluster Analysis.
9. Cosgrove, C et al; Early and nonreversible decrease of CD161++/MAIT cells in HIV infection. Blood 2013, *121*: 951
10. Ruan, X et al. In Vitro O-Antigen Ligase Assay. Methods Mol Biol 2013, *1022*: 185
11. Belcher, J et a; Heme triggers TLR4 signaling leading to endothelial cell activation and vaso-occlusion in murine sickle cell disease. Blood 2014, *123*: 377

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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Date

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