Technical Data Sheet

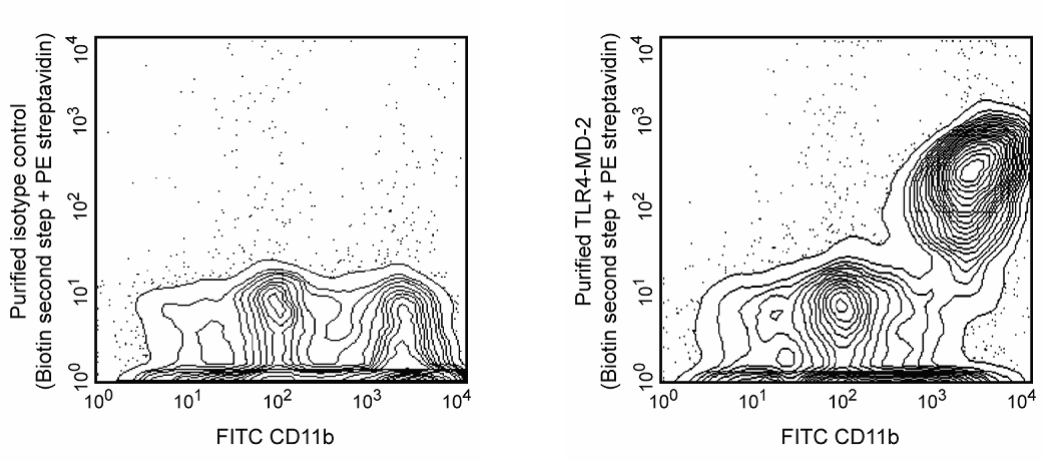
Purified Rat Anti-Mouse CD284/MD-2 Complex

Product Information

Material Number:	**558293**
Alternate Name:	TLR4/MD-2 Complex
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	MTS510
Immunogen:	Mouse Pro-B cell line Ba/F3 expressing transfected BALB/c mouse TLR4 and MD-2
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MTS510 antibody reacts with the molecular complex of Toll-Like Receptor 4 and MD-2 (TLR4-MD-2) which is expressed on LPS responsive macrophages. TLR4, a member of the Toll-Like Receptor Family, has been renamed CD284 and identified to be the transmembrane signal-transducing portion of the receptor for LPS. TLR4 associates on the cell surface with CD14 and MD-2, a 0.7 kDa molecule which is anchored to the membrane via its physical association with TLR4. The association of MD-2 with TLR4 is required for recognition of LPS and the anti-mitotic compound Taxol, which mimics the action of LPS on mouse cells. MTS510 mAb detects TLR4-MD-2 on the surface of thioglycollate-elicited macrophages from all mouse strains tested (ie, BALB/c, C57BL/6, C3H/HeJ, C3H/HeN, and DBA/1), including the C3H/HeJ strain which expresses an LPS-resistant mutant TLR4. Expression of TLR4-MD-2 is down-regulated on peritoneal macrophages after exposure to LPS, correlating with the occurrence of LPS tolerance. TLR4-MD-2 is not detected on splenocytes or thymocytes.



Expression of TLR4-MD-2 on peritoneal macrophages. *Thioglycollate-elicited peritoneal macrophages from BALB/c mice were stained with either purified rat IgG2a, κ isotype control mAb R35-95 (Cat. No. 553927, left panel) or purified mAb MTS510 (right panel), in the presence of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142). Staining was detected by biotinylated anti-rat IgG2a mAb RG7/1.30 (Cat. No. 553894) then Streptavidin-PE (Cat. No. 554061). The macrophages were identified by staining with FITC-conjugated anti-mouse CD11b (Integrin αM chain) mAb M1/70 (Cat. No. 557396/553310). The total viable leukocytes are displayed. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4°C.

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Application Notes

Application

Flow cytometry	Routinely Tested
Immunoprecipitation	Reported
Blocking	Reported

Recommended Assay Procedure:

Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) may help to reduce non-specific binding to cells bearing Fcγ receptors. If Mouse BD Fc Block™ is used, then it is important that the second-step anti-rat IgG antibody does not cross-react with the 2.4G2 mAb (Rat IgG2b, κ); we recommend the use of biotinylated anti-rat IgG2a mAb RG7/1.30 (Cat. No. 553894) with a "bright" third-step reagent, such as Streptavidin-PE (Cat. No. 554061). Other reported applications include immunoprecipitation1, and blocking of LPS-induced NF-κB activation or TNF production.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553927	Purified Rat IgG2a, κ Isotype Control	0.5 mg	R35-95
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553894	Biotin Mouse Anti-Rat IgG2a	0.5 mg	RG7/1.30
554061	PE Streptavidin	0.5 mg	(none)
557396	FITC Rat Anti-Mouse CD11b	0.1 mg	M1/70

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

References

Akashi S, Saitoh S, Wakabayashi Y, et al. Lipopolysaccharide interaction with cell surface Toll-like receptor 4-MD-2: higher affinity than that with MD-2 or CD14. *J Exp Med.* 2003; 198(7):1035-1042.(Clone-specific: Immunoprecipitation)

Akashi S, Shimazu R, Ogata H, et al. Cutting edge: cell surface expression and lipopolysaccharide signaling via the toll-like receptor 4-MD-2 complex on mouse peritoneal macrophages. *J Immunol.* 2000; 164(7):3471-3475.(Immunogen: Blocking, Immunoprecipitation)

Beutler B, Poltorak A. The sole gateway to endotoxin response: how LPS was identified as Tlr4, and its role in innate immunity. *Drug Metab Dispos.* 2001; 29(4):474-478.(Biology)

Kawasaki K, Nogawa H, Nishijima M. Identification of mouse MD-2 residues important for forming the cell surface TLR4-MD-2 complex recognized by anti-TLR4-MD-2 antibodies, and for conferring LPS and taxol responsiveness on mouse TLR4 by alanine-scanning mutagenesis. *J Immunol.* 2003; 170(1):413-420.(Biology)

Nomura F, Akashi S, Sakao Y, et al. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. *J Immunol.* 2000; 164(7):3476-3479.(Immunogen)

Shimazu R, Akashi S, Ogata H, et al. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4.. *J Exp Med.* 1999; 189(11):1777-1782.(Biology)