

Technical Data Sheet

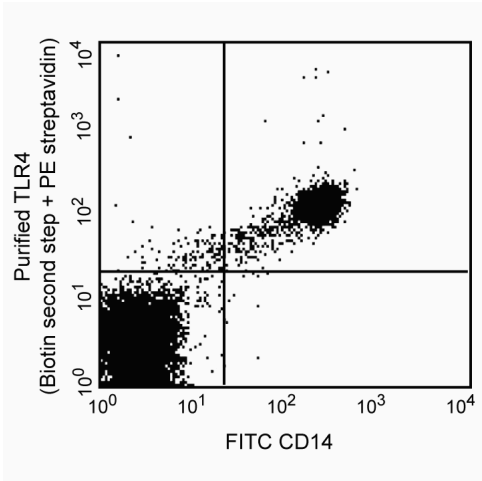
Purified Mouse Anti-Human Toll-Like Receptor 4

Product Information

Material Number:	551964
Alternate Name:	TLR4, CD284
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	HTA125
Immunogen:	Human TLR4-transfected Ba/F3 cell line
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The monoclonal antibody HTA125 reacts with the human Toll-like receptor 4 (TLR4). Toll-like receptors (TLR) are type I transmembrane proteins characterized by an extracellular domain containing leucine-rich repeats and a cytoplasmic domain similar to IL-1R family. In human, ten TLR have been identified so far. The TLR family acts as pattern-recognition receptor is the essential component of pathogen recognition and innate immunity. TLR4 has been identified as the receptor for Gram-negative bacterial LPS. TLR4 mRNA has been found in spleen, placenta, ovary, intestine and lung. Using HTA125, TLR4 protein expression has been detected in monocytes, B cells, and T cells in human peripheral blood. Human TLR4 gene has been mapped to chromosome 9q32. The immunogen used to generate HTA125 hybridoma was human TLR4-transfected Ba/F3 cell line.



Detection of TLR4 expression on human peripheral monocytes by purified anti-human TLR4 antibody HTA125 (Cat. No. 551964). Human PBMC were stained with 0.5 µg of purified HTA125 using 3-step staining protocol outlined below and anti-human CD14-FITC (Cat. No. 555397) and analyzed with a BD FACScan™ Flow Cytometer (Becton Dickinson, San Jose, CA).

Preparation and Storage

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

Application Notes

Application

Flow cytometry	Routinely Tested
Blocking	Reported

Recommended Assay Procedure:

A multistep step staining procedure is recommended to amplify immunofluorescent signals for the flow cytometric analysis of human TLR4 expression:

Step 1: Incubate 10⁶ cells with 0.25 - 1.0 µg/test of purified HTA125 antibody at 4°C for at least 15 - 20 minutes. Wash cells two times with staining medium containing sodium azide (e.g., BD Pharmingen™ Stain Buffer, Cat. No. 554656).

Step 2: Incubate the cells with biotinylated anti-mouse Ig (Cat. No. 553999) or biotinylated anti-mouse IgG2a, (Cat. No. 553388) at 4°C for 20 minutes. Wash cells two times.

Step 3: Incubate the cells with ≤0.06 µg of streptavidin-phycoerythrin (Cat. No. 554061) at 4°C for 20 minutes. Wash two times. Resuspend cells in stain buffer and analyze stained cells by flow cytometry using appropriate specificity and compensation controls.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
555571	Purified Mouse IgG2a, κ Isotype Control	0.1 mg	G155-178
553999	Biotin Goat Anti-Mouse Ig (Multiple Adsorption)	0.5 mg	Polyclonal
554061	PE Streptavidin	0.5 mg	(none)
555397	FITC Mouse Anti-Human CD14	100 tests	M5E2
553388	Biotin Rat Anti-Mouse IgG2a	0.5 mg	R19-15
554656	Stain Buffer (FBS)	500 ml	(none)
555899	Lysing Buffer	100 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.

References

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