

AM26438AF-N**Monoclonal Antibody to CD4 - Azide Free**

Alternate names:	T-cell surface antigen T4/Leu-3, T-cell surface glycoprotein CD4
Quantity:	0.1 mg
Concentration:	1.0 mg/ml
Background:	<p>The T cell receptor for antigen (TCR) is a membrane-bound member of the immunoglobulin superfamily (IgSF) that recognizes a complex of antigen bound to self major histocompatibility complex (MHC) molecules expressed on the cell-surface of antigen-presenting cells (APC). Both cytotoxic and helper T cells typically respond to antigen which is associated with different types of MHC molecules, class I and II, respectively. CD4 is a 55-60 kDa cell-surface glycoprotein, which participates in the molecular complexes involved both in T cell development and its antigen recognizing activity, by binding to the nonpolymorphic region of class II MHC. This interaction results in increased intercellular adhesion and enhanced stimulation of T cells by signal transduction which is mediated by intracellular molecules. CD4 is also considered as a stage marker of T cell development in thymus, for it is expressed on the cell surface in a stage specific manner, during T cell development.</p>
Uniprot ID:	P01730
NCBI:	NP_000607.1
GeneID:	920
Host / Isotype:	Mouse / IgG1
Clone:	4H5
Immunogen:	PHA-activated human CD4+ T-cells
Format:	State: Liquid Ig fraction Purification: Protein A agarose Buffer System: PBS containing 50% glycerol, pH 7.2. No preservative is contained.
Applications:	Flow cytometry: 5 µg/ml (final concentration). For details see protocol below. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antibody reacts with CD4 antigen.
Species Reactivity:	Tested: Human
Add. Information:	This product was originally produced by MBL International.
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
General Readings:	1. Ravichandran, K. S., et al., Microbiol. Immunol. 205, 47-62 (1996). 2. Leahy, D. J., FASEB J. 9, 17-25 (1995).
Protocols:	Flow cytometric analysis for whole blood cells We usually use Falcon tubes or equivalents as reaction tubes for all steps described below. 1) Add 50 µL of CD4 monoclonal antibody (4H5) at the concentration as suggest in the

APPLICATIONS diluted in the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃] into each tube.

- 2) Add 50 μ L of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 oC).
- 3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Add 30 μ L of 1:100 FITC conjugated anti-mouse IgG diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 6) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
- 7) Add 1 mL of H₂O to each tube and incubate for 10 minutes at room temperature.
- 8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 10) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; lymphocyte, monocyte)

Pictures:

Flow cytometric analysis of CD4 antigen expression on human lymphocyte (left) and human monocyte (right). Open histograms indicate the reaction of Isotypic control to the cells. Shaded histograms indicate the reaction of AM26438AF-N to the cells.

