Human CTLA-4 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF-386-PB

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CTLA-4 in direct ELISAs and Western blots. In Western blots, approximately 25% cross-reactivity with recombinant mouse CTLA-4 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*S. frugiperda* insect ovarian cell line *Sf* 21-derived recombinant human CTLA-4 Ala37-Phe162 Accession # Q6GR94
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

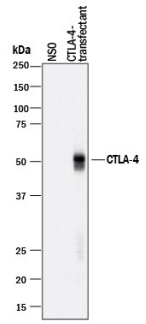
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

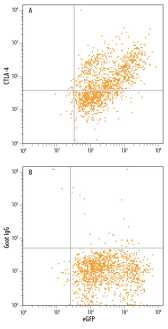
DATA

Western Blot



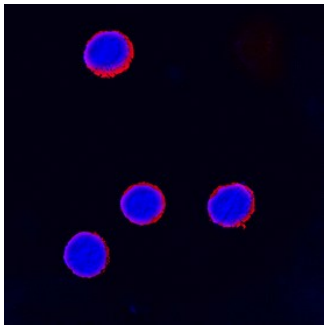
Detection of Human CTLA-4 by Western Blot. Western blot shows lysates of NS0 mouse myeloma cell line either mock transfected or transfected with human CTLA-4. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human CTLA-4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-386-PB) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for CTLA-4 at approximately 50 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of CTLA-4 in NS0 Mouse Cell Line Co-transfected with CTLA-4 and eGFP by Flow Cytometry. NS0 mouse myeloma cell line co-transfected with human CTLA-4 and eGFP was stained with either (A) Goat Anti-Human CTLA-4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-386-PB) or (B) Normal Goat IgG Control (Catalog # AB-108-C) followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108).

Immunocytochemistry



CTLA-4 in Human Peripheral Blood Mononuclear Cells. CTLA-4 was detected in immersion fixed human peripheral blood mononuclear cells treated with treated with PMA and calcium ionomycin using Goat Anti-Human CTLA-4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-386-PB) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

CTLA-4 and CD28, together with their ligands B7-1 and B7-2, constitute one of the dominant costimulatory pathways that regulate T- and B-cell responses. CTLA-4 and CD28 are structurally homologous molecules that are members of the immunoglobulin (Ig) gene superfamily. Both CTLA-4 and CD28 are composed of a single Ig V-like extracellular domain, a transmembrane domain and an intracellular domain. CTLA-4 and CD28 are both expressed on the cell surface as disulfide-linked homodimers or as monomers. The genes encoding these two molecules are closely linked on human chromosome 2. CTLA-4 was originally identified as a gene that was specifically expressed by cytotoxic T lymphocytes. However, CTLA-4 transcripts have since been found in both Th1 and Th2, and $CD4^+$ and $CD8^+$ T cell clones. Whereas CD28 expression is constitutive on the surfaces of 95% of $CD4^+$ T cells and 50% of $CD8^+$ T cells and is down regulated upon T cell activation, CTLA-4 expression is upregulated rapidly following T cell activation and peaks approximately 24 hours following activation. Although both CTLA-4 and CD28 can bind to the same ligands, CTLA-4 binds to B7-1 and B7-2 with 20-100-fold higher affinity than CD28. The physiological role of CTLA-4 in T cell costimulation is currently being studied.

References:

1. Lenschow, D.J. *et al.* (1996) Annu. Rev. Immunol. **14**:233.
2. Hathcock, K.S. and R.J. Hodes (1996) Advances in Immunol. **62**:131.
3. Ward, S.G. (1996) Biochem. J. **318**:361.

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