

**DESCRIPTION**

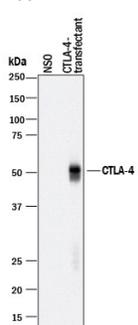
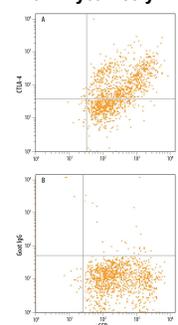
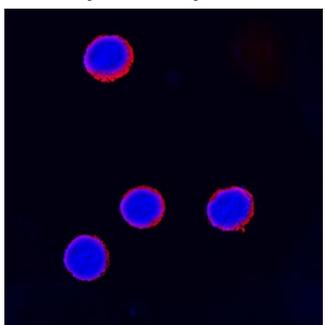
<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human CTLA-4 in direct ELISAs and Western blots. In Western blots, approximately 25% cross-reactivity with recombinant mouse CTLA-4 is observed.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>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

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

**DATA**

<p><b>Western Blot</b></p>  <p><b>Detection of Human CTLA-4 by Western Blot.</b> 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.</p>	<p><b>Flow Cytometry</b></p>  <p><b>Detection of CTLA-4 in NS0 Mouse Cell Line Co-transfected with CTLA-4 and eGFP by Flow Cytometry.</b> 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).</p>
<p><b>Immunocytochemistry</b></p>  <p><b>CTLA-4 in Human Peripheral Blood Mononuclear Cells.</b> 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 <a href="#">Fluorescent ICC Staining of Non-adherent Cells</a>.</p>	

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

## 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<sup>+</sup> and CD8<sup>+</sup> T cell clones. Whereas CD28 expression is constitutive on the surfaces of 95% of CD4<sup>+</sup> T cells and 50% of CD8<sup>+</sup> 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.