

DESCRIPTION

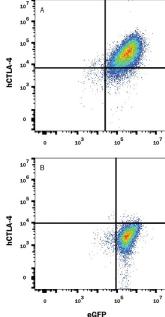
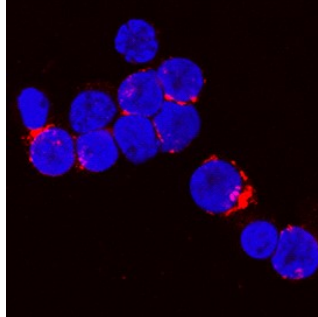
Species Reactivity	Human
Specificity	Detects human CTLA-4 in direct ELISAs.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2188A
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human CTLA-4 Ala37-Phe162 Accession # P16410
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 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.

	Recommended Concentration	Sample
Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
Immunocytochemistry	3-25 µg/mL	See Below

DATA

<p>Flow Cytometry</p>  <p>Detection of CTLA-4 in NS0 Mouse Cell Line Transfected with Human CTLA-4 and eGFP by Flow Cytometry. NS0 mouse myeloma cell line transfected with (A) human CTLA-4 or (B) irrelevant transfectants and eGFP was stained with Rabbit Anti-Human CTLA-4 Monoclonal Antibody (Catalog # MAB3252) followed by APC-conjugated Goat anti-Rabbit IgG Secondary Antibody (Catalog # F0111). Quadrants were set based on Normal Rabbit IgG Control Antibody (Catalog # MAB1050, data not shown). View our protocol for Staining Membrane-associated Proteins.</p>	<p>Immunocytochemistry</p>  <p>CTLA-4 in Human PBMCs. CTLA-4 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Rabbit Anti-Human CTLA-4 Monoclonal Antibody (Catalog # MAB3252) at 3 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.</p>
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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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. <ul style="list-style-type: none"> • 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.

BACKGROUND

CTLA-4 (cytotoxic T-lymphocyte associated protein-4, designated CD152), is a type I transmembrane T cell inhibitory molecule that is a member of the Ig superfamily (1, 2). Human or mouse CTLA-4 cDNA encodes 223 amino acids (aa) including a 35 aa signal sequence, a 126 aa extracellular domain (ECD) with one Ig-like V-type domain, a 21 aa transmembrane (TM) sequence, and a 41 aa cytoplasmic sequence. It is found as a covalent homodimer of 41-43 kDa (2). Within the ECD, human CTLA-4 shares 68%, 71% and 83-86% aa sequence identity with mouse, rat and porcine/bovine/rabbit/feline/canine CTLA-4, respectively. A 174 aa form that lacks TM and cytoplasmic sequences (sCTLA-4) is possibly secreted (3-5). Isoforms of 56-79 aa that mainly contain parts of the cytoplasmic domain are reported. In mouse, an isoform lacking the Ig-like domain has ligand-independent inhibitory activity and is termed liCTLA-4 (6). CD28, which is structurally related to CTLA-4, is constitutively expressed on naïve T cells and promotes T cell activation when engaged by B7-2 on antigen-presenting cells (APC) within the immunological synapse (IS) (1, 7, 8). In contrast, CTLA-4 is recruited from intracellular vesicles to the IS beginning 1-2 days after T cell activation (2, 7, 8). It forms a linear lattice with B7-1 on APC, inducing negative regulatory signals and ending T cell activation (9). Abatacept, a therapeutic human CTLA-4-Ig fusion protein (trade name Orencia), competes with CD28 for B7-1 and B7-2 binding and has been used to antagonize T cell activation in autoimmune conditions and to enhance transplant survival (10). Mice deleted for CTLA-4 show no abnormalities until after birth, but then develop lethal autoimmune reactions due to continued T cell activation and poor control by regulatory T cells, which constitutively express CTLA-4 in wild-type mice and humans (11-13).

References:

1. Harper, K. *et al.* (1991) *J. Immunol.* **147**:1037.
2. Teft, W.A. *et al.* (2006) *Annu. Rev. Immunol.* **24**:65.
3. Magistrelli, G. *et al.* (1999) *Eur. J. Immunol.* **29**:3596.
4. Tector, M. *et al.* (2009) *BMC Immunol.* **10**:51.
5. Oaks, M.K. and K.M. Hallett (2000) *J. Immunol.* **164**:5015.
6. Vijayakrishnan, L. *et al.* (2004) *Immunity* **20**:563.
7. Pentcheva-Hoang, T. *et al.* (2004) *Immunity* **21**:401.
8. Jansson, A. *et al.* (2005) *J. Immunol.* **175**:1575.
9. Darlington, P.J. *et al.* (2005) *J. Immunol.* **175**:996.
10. Platt, A.M. *et al.* (2010) *J. Immunol.* **185**:1558.
11. Wing, K. *et al.* (2008) *Science* **322**:271.
12. Friedline, R.H. *et al.* (2009) *J. Exp. Med.* **206**:421.
13. Jain, N. *et al.* (2010) *Proc. Natl. Acad. Sci. USA* **107**:1524.