

## DESCRIPTION

<b>Species Reactivity</b>	Human/Mouse
<b>Specificity</b>	Detects human CTLA-4 in direct ELISAs. Detects human and mouse CTLA-4 in Western blots.
<b>Source</b>	Monoclonal Mouse IgG <sub>1</sub> Clone # 1003705
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	Chinese Hamster Ovary cell line, CHO-derived human CTLA-4 Ala37-Phe162 Accession # P16410
<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.

	Recommended Concentration	Sample
<b>Western Blot</b>	2 µg/mL	See Below
<b>Agonist Activity</b>	Measured by its ability to inhibit anti-CD3 antibody induced IFN-γ secretion by human peripheral blood mononuclear cells (PBMC). The ED <sub>50</sub> for this effect is 0.05-0.4 µg/mL.	

## DATA

**Western Blot**

**Detection of Human and Mouse CTLA-4 by Western Blot.** Western blot shows lysates of NS0 mouse myeloma cell line transfected with Human CTLA-4 and mouse lymph node tissue. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human/Mouse CTLA-4 Monoclonal Antibody (Catalog # MAB386) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for CTLA-4 at approximately 52 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Agonist Activity**

**Inhibition of Anti-CD3 Induced IFN-γ Secretion by Human/Mouse CTLA-4 Antibody.** Anti-Human/Mouse CTLA-4 Monoclonal Antibody (Catalog # MAB386) inhibits IFN-γ secretion in human peripheral blood mononuclear cells (PBMC) induced by immobilized Mouse Anti-Human CD3e Monoclonal Antibody (Catalog # MAB100) coated at 1 µg/mL (100 µL/well) in a dose-dependent manner (green line) as measured by the Human IFN-γ Quantikine ELISA Kit (Catalog # DIF50C). The ED<sub>50</sub> is typically 0.05-0.4 µg/mL.

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.5 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 (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:**

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