

DESCRIPTION

Species Reactivity	Human
Specificity	Detects a synthetic peptide specific for human CTLA-4 around amino acid 150 in Direct ELISA.
Source	Monoclonal Mouse IgG _{2B} Clone # 1121235
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Synthetic Peptide Accession # P16410
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

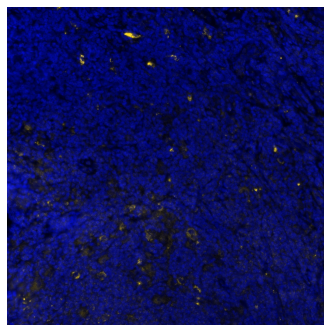
APPLICATIONS

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
Immunohistochemistry	0.25-25 µg/mL	Immersion fixed paraffin-embedded sections of human tonsil
COMET	3 µg/mL	Immersion fixed paraffin-embedded sections of human Hodgkin Lymphoma, Spleen and Tonsil

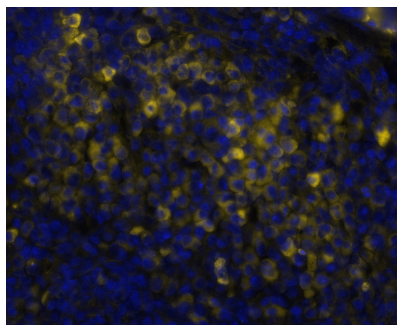
DATA

Multiplex Immunofluorescence



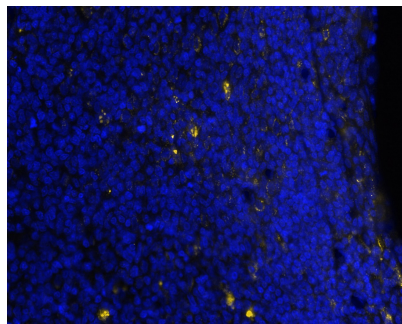
CTLA4 in Human Hodgkin Lymphoma via seqIF™ staining on COMET™ CTLA4 was detected in immersion fixed paraffin-embedded sections of human Hodgkin Lymphoma using Mouse Anti-Human CTLA4, Monoclonal Antibody (Catalog # MAB11809) at 3 µg/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; EpreDia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ 647 Goat anti-Mouse IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647MS) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane. Protocol available in [COMET™ Panel Builder](#).

Multiplex Immunofluorescence



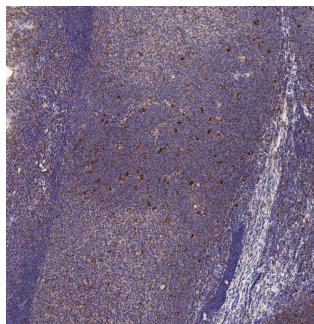
CTLA4 in Human Spleen via seqIF™ staining on COMET™ CTLA4 was detected in immersion fixed paraffin-embedded sections of human spleen using Mouse Anti-Human CTLA4, Monoclonal Antibody (Catalog # MAB11809) at 3µg/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; EpreDia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ 647 Goat anti-Mouse IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647MS) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane. Protocol available in [COMET™ Panel Builder](#).

Multiplex Immunofluorescence



CTLA4 in Human Tonsil via seqIF™ staining on COMET™ CTLA4 was detected in immersion fixed paraffin-embedded sections of human tonsil using Mouse Anti-Human CTLA4, Monoclonal Antibody (Catalog # MAB11809) at 3µg/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; EpreDia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ 647 Goat anti-Mouse IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647MS) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane. Protocol available in [COMET™ Panel Builder](#).

Immunohistochemistry



Immersion fixed paraffin-embedded sections of human tonsil CTLA-4 was detected in immersion fixed paraffin-embedded sections of human tonsil using Mouse Anti-Human CTLA-4 Monoclonal Antibody (Catalog # MAB11809) at 5 µg/ml overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using the HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # HAF007) and counterstained with hematoxylin (blue). Specific staining was localized to the membrane. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2 mg/ml in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
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.

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. Recombinant human CTLA-4 Fc chimera preparations produced at R&D Systems have been shown to bind both B7-1 and B7-2 with high affinity and to inhibit CD 28 signalling competitively.

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.