# CTLA-4

Concentrated and Prediluted Monoclonal Antibody
901-3211-101717

BIOCARE MEDICAL

| | | |
|---|---|---|
| **Catalog Number:** | **ACI 3211 A, B** | **API 3211 AA** |
| **Description:** | 0.1, 0.5 ml, concentrated | 6.0 ml, prediluted |
| **Dilution:** | 1:100 | Ready-to-use |
| **Diluent:** | Da Vinci Green | N/A |

**Intended Use:**
For In Vitro Diagnostic Use
CTLA-4 [UMAB249] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of CTLA-4 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

**Summary and Explanation:**
Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) is a member of the CD28 superfamily and is a negative regulator of T cell-mediated immune responses. CTLA-4 exhibits cell surface and intracellular constitutive expression on memory T-cells and at a low level by T-regulatory cells (Tregs; 2-4% of circulating CD4+ T cells) (1,2). CTLA-4 primarily inactivates T-cell activity by competing with the CD28 costimulatory molecule (3). CD28 and CTLA-4 share the identical ligands of CD80 and CD86 on antigen-presenting cells; and thus CTLA-4 competes with CD28 function in T-cell survival, proliferation, and recruitment (3,4). In particular, CTLA-4 down-modulates CD4+ helper T-cell activity and enhances Treg immunosuppressive functions (5,6).
CTLA-4 has been shown to play a role in human diseases (1). CTLA-4 acts as a physiological brake on the activated immune system in order to maintain immune homeostasis. Several suppressive mechanisms for T-cell functions have been attributed to CTLA-4. FDA approved Ipilimumab (IgG1 isotype), a monoclonal antibody to CTLA-4, was the first immunotherapeutic drug directed toward CTLA-4 inhibition to demonstrate overall survival benefit in metastatic melanoma (1,7). Another CTLA-4 inhibitor, tremelimumab (IgG2 isotype), has also proven successful in metastatic melanoma and other malignancies (1,7).

**Principle of Procedure:**
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal
**Species Reactivity:** Human
**Clone:** UMAB249
**Isotype:** IgG1
**Total Protein Concentration**: ~10 mg/ml. Call for lot specific Ig concentration.
**Epitope/Antigen:** Full length human recombinant protein of human CTLA-4
**Cellular Localization:** Cell membrane/cytoplasm
**Positive Tissue Control:** Tonsil
**Known Applications:**
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
**Supplied As:** Buffer with protein carrier and preservative

**Storage and Stability:**
Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

**Protocol Recommendations:**
**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidazed 1.
**Pretreatment:** Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.
**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Biocare's Background Punisher.
**Primary Antibody:** Incubate for 30 minutes at RT.
**Probe:** Incubate for 10 minutes at RT with a secondary probe.
**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.
**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Biocare's Warp Red.
**Counterstain:**
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
**Technical Note:**
This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.
**Performance Characteristics:**
Sensitivity and specificity on diseased tissue and tissue cross-reactivity on normal tissue is summarized in Tables 1 and 2, respectively.
**Limitations:**
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.
**Quality Control:**
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011
**Precautions:**
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide ($NaN_3$) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (8)

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

Rev: 062117

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

**Precautions Cont'd:**
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (9)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

**Troubleshooting:**
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

**References:**
1. Buchbinder EI, McDermott DF. Cytotoxic T-lymphocyte antigen-4 blockade in melanoma. Clinical Therapeutics. 2015; 37:755-63.
2. Baecher-Allan C, *et al*. Human CD4+CD25+ regulatory T cells. Semin Immunol. 2004 Apr; 16(2):89-98.
3. Schwartz RH. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell. 1992; 71:1065-8.
4. Azuma M, *et al*. B70 antigen is a second ligand for CTLA-4 and CD28. Nature 1993; 366:76-9.
5. Hathcock KS, *et al*. Identification of an alternative CTLA-4 ligand costimulatory for T cell activation. Science. 1993; 262:905-7.
6. Wing K, *et al*. CTLA-4 control over Foxp3+ regulatory T cell function. Science. 2008; 322:271-5.
7. Shin DS, Ribas A. The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? Curr Opin Immunol. 2015; 33:23-35.
8. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
9. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

**Table 1:** Sensitivity and specificity was determined by testing formalin-fixed, paraffin-embedded diseased tissues.

| Tissue | Positive Cases | Total Cases |
|---|---|---|
| Bladder Cancer | 0 | 8 |
| Breast Cancer | 0 | 11 |
| Colon Cancer | 3 | 17 |
| Lung Cancer | 0 | 30 |
| Ovarian Cancer | 0 | 10 |
| Prostate Cancer | 0 | 15 |
| Melanoma | 1 | 7 |

**Table 2:** Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

| Tissue | Positive Cases | Total Cases |
|---|---|---|
| Cerebellum | 0 | 1 |
| Cerebral Cortex | 0 | 1 |
| Pituitary | 0 | 1 |
| Adrenal Gland | 0 | 1 |
| Thymus | 1 | 1 |
| Tonsil | 1 | 1 |
| Thyroid | 0 | 1 |
| Esophagus | 0 | 1 |
| Stomach | 0 | 1 |
| Small Intestine | 1 | 1 |
| Colon | 1 | 1 |
| Appendix | 1 | 1 |
| Pancreas | 0 | 1 |
| Spleen | 1 | 1 |
| Ovary | 0 | 1 |
| Cervix | 0 | 1 |
| Endometrium | 0 | 1 |
| Fallopian tube | 0 | 1 |
| Placenta | 0 | 1 |
| Kidney | 0 | 1 |
| Bladder | 0 | 1 |
| Urethra | 0 | 1 |
| Breast | 0 | 1 |
| Prostate | 0 | 1 |
| Testis | 0 | 1 |
| Myocardium | 0 | 1 |
| Smooth Muscle | 0 | 1 |
| Skeletal Muscle | 0 | 1 |
| Lymph Node | 1 | 1 |
| Aorta | 0 | 1 |
| Bone Marrow | 0 | 1 |
| Lung | 0 | 1 |
| Skin | 0 | 1 |
| Liver | 0 | 1 |