

# CTLA-4 [CAL49]

Concentrated and Prediluted Rabbit Monoclonal Antibody  
901-3220-071718

**BIOCARE**  
M E D I C A L

<b>Catalog Number:</b>	<b>ACI 3220 A, B</b>	<b>API 3220 AA</b>
<b>Description:</b>	0.1, 0.5, ml concentrated	6.0 ml, prediluted
<b>Dilution:</b>	1:100	Ready-to-use
<b>Diluent:</b>	Da Vinci Green	N/A

## Intended Use:

For In Vitro Diagnostic Use

CTLA-4 [CAL49] is a rabbit 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 expression is induced on the surface of T cells after CD28 binding and activation, and is constitutively expressed on T-regulatory cells, acting as an immune checkpoint inhibitor, down-regulating T cell activity. (1-3). CTLA-4 primarily inactivates T-cell activity by competing with the CD28 costimulatory molecule (1,3). 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).

CTLA-4 has been shown to play a role in human diseases (1,3). 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 (1,6). Another CTLA-4 inhibitor, tremelimumab (IgG2 isotype), has also proven successful in metastatic melanoma and other malignancies (1,6).

## 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, an enzyme labeled polymer is added to bind to the primary antibody. This detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Rabbit monoclonal

**Species Reactivity:** Human and mouse

**Clone:** CAL49

**Isotype:** IgG1

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration.

**Epitope/Antigen:** Synthetic peptide derived from a region of the CTLA-4 protein

**Cellular Localization:** Membranous/cytoplasmic

**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 2°C to 8°C. 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 2°C to 8°C.

## Protocol Recommendations (intelliPATH and manual use):

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Biocare's Borg Decloaker. Refer to the Borg 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:** N/A

**Polymer:** Incubate for 30 minutes at RT with a secondary-conjugated 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.

## Protocol Recommendations (Ventana BenchMark ULTRA Slide Staining System):

API3220 is compatible for use with the Ventana BenchMark ULTRA Slide Staining System. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

**Template/Detection:** OptiView DAB IHC

**Pretreatment Protocol:** CC1 64 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

**Primary Antibody:** 32 minutes, 36°C

## Technical Note:

This antibody, for intelliPATH and manual use, has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

## Performance Characteristics:

Sensitivity, specificity and cross-reactivity are 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<sub>3</sub>) used as a preservative is toxic if

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## Precautions Cont'd:

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) (7)

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. (8)

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. Scalapino KJ, Daikh DI. CTLA-4: a key regulatory point in the control of autoimmune disease. *Immunol Rev*. 2008 Jun;223:143-55.
4. 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.
5. Wing K, *et al.* CTLA-4 control over Foxp3+ regulatory T cell function. *Science*. 2008; 322:271-5.
6. 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.
7. 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."
8. 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.

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

Tissue*	Positive Cases	Total Cases
Bladder Cancer	7	11
Breast Cancer	7	8
Colon Cancer	11	13
Kidney Cancer	3	7
Lung Cancer	15	16
Melanoma	3	3
Ovarian Cancer	4	11
Prostate Cancer	3	12

\*Some tumor cells are stained with this antibody

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

Tissue	Positive Cases	Total Cases
Adrenal Gland	0	1
Thymus	1	1
Tonsil	4	4
Thyroid	0	1
Esophagus	0	1
Stomach	0	1
Small Intestine	1	1
Colon	0	1
Appendix	0	1
Pancreas	0	1
Spleen	1	1
Ovary	0	1
Cervix	0	1
Endometrium	0	1
Placenta	0	1
Kidney	9	9
Bladder	0	1
Breast	0	1
Prostate	1	4
Testis	1	1
Myocardium	0	1
Smooth Muscle	0	1
Lymph Node	1	1
Aorta	0	1
Lung	0	1
Skin	0	1
Liver	2	2