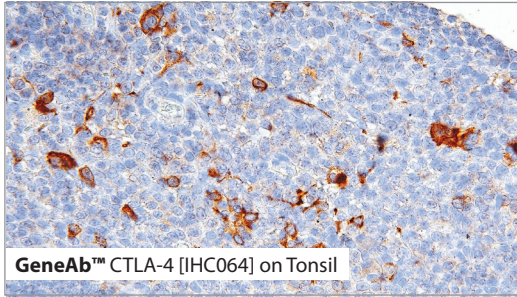




GeneAb™ Monoclonal Rabbit Anti-Human

CTLA-4 Antibody

Clone: IHC064**Positive Control:** Tonsil**Source:** Rabbit Monoclonal**Localization:** Membranous, Cytoplasmic

Product Information

REF	Description
IHC064-100	0.1 ml, Concentrate
IHC064-1	1 ml, Concentrate
IHC064-7	7 ml, Predilute
IHC064-PC	3 Positive Control Slides

1. Intended Use

This antibody is intended for Research Use Only.

The CTLA-4 [IHC064] antibody is intended for qualified laboratories to qualitatively identify by light microscopy the presence of associated antigens in sections of formalin-fixed, paraffin-embedded tissue sections using IHC test methods. Use of this antibody is indicated as an aid in the identification of CTLA-4 strictly for research purposes.

2. Summary and Explanation

Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA-4) is a receptor on T helper cells that functions as an immune checkpoint and downregulator of immune responses. Mutations in CTLA-4 are associated with insulin-dependent diabetes mellitus, Hashimoto's thyroiditis, Graves' disease, systemic lupus erythematosus (SLE), celiac disease, primary biliary cirrhosis, thyroid-associated orbitopathy, multiple sclerosis, and other autoimmune diseases. The spliced variant of CTLA-4 in SLE is present in the patient's serum. Haploinsufficiency of CTLA-4 causes the immune system disorder known as CTLA-4 deficiency or CHAI disease (CTLA-4 haploinsufficiency with autoimmune infiltration).

3. Principles and Procedures

Visualization of the antigen present in tissue sections is accomplished in a multi-step immunohistochemical staining process, in conjunction with a horseradish peroxidase (HRP) or alkaline phosphatase (AP) linked detection system. The process involves the addition of the stated antibody (primary antibody) to a tissue slide, followed by a secondary antibody (linked to an enzyme complex) which specifically binds to the primary antibody. A chromogenic substrate is then added which reacts with the enzyme complex, resulting in a colorimetric reaction at the site of the antigen. Results are interpreted using a light microscope.

4. Materials and Methods

Product Format	Dilution	Buffer Composition
Predilute	Ready to Use	GenomeMe Antibody Diluent (Cat# IHC000)
Concentrate	1:25-1:100	Tris Buffer, pH 7.3 - 7.7, with 1% BSA and <0.1% Sodium Azide

Reconstitution, Mixing, Dilution, and Titration

The prediluted antibody does not require any mixing, dilution, reconstitution, or titration; the antibody is ready-to-use and optimized for staining. Any further dilution may affect the quality of the staining signal or antibody-antigen interaction.

The concentrated antibody requires dilution using an Antibody Diluent Buffer, to the recommended working dilution range listed in the table above, prior to use.

Storage and Handling

Store at 2-8°C.

To ensure stability, immediately replace vial back in the refrigerator after each use. When stored correctly, the antibody is stable until the expiry date indicated on the label.

Positive and negative controls should be concurrently run with tissue specimens, to enable identification of any inadequacies with the antibody or reagents. If antibody stability issues are suspected, please contact GenomeMe Customer Service at info@genomeme.ca.

Specimen Collection and Preparation for Analysis

Each tissue section should be fixed with 10% neutral buffered formalin, cut to the applicable thickness (4µm), and placed on a glass slide that is positively charged. The prepared slide should then be baked for a minimum of 30 minutes in a 53-65°C oven (do not exceed 24 hours).

Note: Performance evaluation has been shown on human tissues only. Variable results may occur due to extended fixation time or variations in tissue preparation.

Materials Required But Not Provided

The following materials are required but are not provided:

- a) Detection system (ie. BOND Polymer Refine Detection Kit or UltraView Universal DAB Detection Kit)
- b) Chromogen (ie. DAB Substrate Kit)
- c) IHC wash buffer and blocking solution
- d) Hematoxylin or other counterstaining reagents
- e) Ethanol or reagent alcohol, xylene or xylene substitute and mounting medium
- f) Antibody diluents
- g) Positive and negative control tissue

5. Instructions For Use

Recommended Staining Protocols for the CTLA-4 [IHC064] antibody:

Automated Staining with Leica Biosystems Bond-MAX Platform:

This primary antibody has been optimized and validated using the Leica Bond-MAX Fully Automated IHC & ISH Stainer, applying IHC Protocol F. Antibody concentrate dilution range is 1:25-1:100.

The following edits are recommended for the protocol:

- a) Marker Incubation Time: 30 minutes
- b) Heat-induced epitope retrieval (HIER) is recommended using Leica Bond ER Solution 2 for 30 minutes.
- c) Rearrange the "Peroxide Block" step to occur after "Polymer" but before "Mixed DAB Refine" step.

For all other automated IHC staining systems, refer to the corresponding user manual for specific instructions.

Manual Staining:

1. **Pretreatment:** Perform heat-induced epitope retrieval (HIER) at pH 6 or 9 for 10 to 30 minutes.
2. **Blocking:** If HRP is used, block with peroxidase blocking solution for 10-15 minutes at room temperature. Replace with alkaline phosphatase blocking solution if an AP system is used.
3. **Primary Antibody:** Apply and incubate antibody for 30-60 minutes at room temperature or overnight at 4°C.
4. **Secondary Antibody:** Apply and incubate for 20-30 minutes at room temperature.
5. **Substrate Development:** Apply and incubate with DAB or Fast Red for 5-10 minutes at room temperature.
6. **Counterstaining:** Counterstain with hematoxylin for 0.5-5 minutes, based on the hematoxylin used. Rinse with distilled water and bluing solution for 30 seconds.
7. Dehydrate and apply coverslip.

6. Quality Control Procedures and Interpretation of Results

The immunohistochemical staining process results in a colorimetric reaction at the site of the antigen, localized by the primary antibody. The tissue specimen result should be interpreted only after the positive and negative control tissues have been analyzed. It is recommended to include a set of tissue controls with each staining run to monitor for antibody, tissue, and reagent performance.

Tissue sections may contain both positive and negative staining elements. In these cases and where applicable, these sections may serve as both the positive and negative tissue control.

Positive Control Tissue

A positive control tissue should be processed in the same manner as the specimen and run with each test condition to provide control for variables such as tissue processing, fixation, and staining. It should function to provide validity to the specimen results obtained and can consist of fresh autopsy, biopsy, or surgical tissue.

Once stained, the positive control tissue should be analyzed first to ensure that the antibody and all reagents are performing as intended. Counterstaining will result in a blue coloration, which may range from pale to dark depending on the length of the incubation time and potency of the hematoxylin. If positive staining is not observed, the positive control tissue must be deemed invalid and the results obtained with the tissue specimen must also be treated as such.

Negative Control Tissue

Some tissue sections can also function as an internal negative control due to the diversity of staining elements present. This, however, should first be confirmed by the user. Tissue components that do not stain should demonstrate an absence of specific staining. If specific staining is observed, the negative control tissue must be deemed invalid and the results obtained with the tissue specimen must also be treated as such.

Tissue Specimens

Tissue specimens should only be analyzed after the positive and negative control tissues have been deemed valid. Negative staining indicates that the antigen was not detected in the tissue while positive staining represents the presence of the antigen.

A tissue section stained with hematoxylin and eosin should be used to analyze the morphology of the tissue specimen.

Performance Characteristics

This antibody has been validated by immunohistochemistry using a FFPE human tissue microarray comprised of different types of normal and cancerous tissues. Positive staining was observed in tonsil, lymph node and thymus tissues. No staining was seen in skeletal muscle and brain tissues. A representative positive staining image is shown on Page 1.

7. Troubleshooting

1. If tissue sections wash off the slide, this may be caused by:
 - a) Slides are not positively charged.
 - b) Inadequate neutral-buffering of the formalin used for the fixation process.
 - c) A thick tissue section.
 - d) Inadequate drying of the tissue section prior to staining.
2. If the positive control tissue exhibits negative staining, this may be due to:
 - a) An issue with the primary antibody or one of the secondary reagents.
 - b) Improper collection, fixation or deparaffinization of the tissue section.
 - c) Errors in the IHC staining process.
3. If the positive control tissue exhibits weaker staining than expected, this may be due to sub-optimal IHC conditions, partial degradation of the primary antibody or improper storage of secondary reagents. Analysis of the positive and/or negative control tissues can help with determining the cause.

For assistance with all other types of inquiries, please contact GenomeMe Customer Service at **info@genomeme.ca**.

8. Limitations

1. This antibody is intended for Research use Only by qualified laboratory personnel.
2. Due to biological variability inherent to the expression of certain antigens and immunohistochemical procedures, appropriate positive and negative controls should be used alongside the tissue specimen.
3. This antibody, when used with the appropriate detection systems and reagents, detects antigen(s) that remain intact through the tissue fixation, processing and sectioning as described. Any deviation from these recommended procedures or improper handling may compromise the validity and/or analysis of the results.
4. GenomeMe provides prediluted antibodies in a ready-to-use, optimally diluted format for use as instructed. Due to the potential for variation in tissue processing and fixation, it may be necessary to adjust the incubation time of the primary antibody for different tissue specimens.
5. GenomeMe provides concentrated antibodies in a format that requires dilution with GenomeMe Antibody Diluent. Use of a diluent different than that specified in the package insert must be validated by the user to ensure proper compatibility with the antibody.
6. Any discrepancies or unexplained results in control or tissue specimens can be reported to GenomeMe Customer Service at info@genomeme.ca for further assistance. Please refer to the troubleshooting section for common causes of issues.
7. False positive results may occur in tissue specimens due to the possibility of non-immunological binding of substrate reaction products or proteins. False positive results may also occur subject to the type of immunostaining technique used, or due to the activity of pseudoperoxidase, endogenous peroxidase, or endogenous biotin.
8. Due to the effect of autoantibodies or natural antibodies, normal sera from an animal source that is the same as the secondary antisera may result in false negative or false positive results when used in blocking steps.
9. Non-specific staining with horseradish peroxidase may be observed when using tissues containing hepatitis B surface antigen due to the patient's infection with the hepatitis B virus.

9. Warnings and Precautions

1. Ensure proper reagent handling procedures are followed. Always wear laboratory coats, use disposable gloves and other appropriate personal protective equipment when handling reagents.
2. Do not ingest any antibody or reagent. Avoid contact with eyes and other mucous membranes. Should any contact occur, rinse the area with copious amounts of water and follow laboratory procedures for reporting the exposure.
3. All incubation times and temperatures must be validated by the user with first use. Any usage or storage conditions different than that specified on the package insert should also be validated by the user.
4. Treat all tissue specimens, autopsy/biopsy/surgical samples and any materials in contact with these as potentially biohazardous materials and handle with appropriate laboratory precautions.
5. To ensure antibody stability and the accuracy of results, ensure microbial contamination of the antibody does not occur.

10. References

1. Denizot F, et al. *Nature*. 1987; 328:267-70.
2. Dariavach P, et al. *Eur J Immunol*. 1988; 18:1901-5.
3. Krummel MF, et al. *J Exp Med*. 1995; 182:459-65.
4. Kuehn HS, et al. *Science*. 2014; 345:1623-7.
5. Walunas TL, et al. *J Exp Med*. 1996; 183:2541-50.