FOXP3 [86D]

Concentrated and Prediluted Monoclonal Antibody
901-3197-090817

BIOCARE MEDICAL

Catalog Number:	**ACI 3197 A, C**	**API 3197 AA**
Description:	0.1, 1.0 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
FOXP3 [86D] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of FOXP3 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:
FOXP3 is a forkhead transcription factor family member involved in T-cell regulation, activation and differentiation. FOXP3 has been shown to be a master control gene for the development and function of CD4+/CD25+ regulatory T-cells. In IHC, FOXP3 has been shown to be a specific marker for adult T-cell leukemia/lymphoma (1). In melanoma and in breast and lung cancers, high numbers of circulating regulatory T cells have been associated with disease progression (2-5). Conversely, the infiltration of FOXP3+ regulatory T cells into invasive tumors has also been reported to be associated with survival in a variety of cancers (2-7). In colon cancers, a high frequency of FOXP3+ infiltrates has shown to be a positive indicator (6-7). Patients with high FOXP3 expression in Crohn's disease have shown a better response to infliximab therapy (8). In allography recipients, FOXP3 cell levels may also be useful in improving post-transplant management (9).

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; others not tested
Clone: 86D
Isotype: IgG1
Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.
Epitope/Antigen: FOXP3 fusion protein
Cellular Localization: Nuclear
Positive Tissue Control: Colon cancer and 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 is summarized in Table 1.

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

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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) (10)
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 into contact with sensitive areas, wash with copious amounts of water. (11)
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. Roncador G, *et al*. FOXP3, a selective marker for a subset of adult T-cell leukaemia/lymphoma. Leukemia. 2005 Dec; 19(12):2247-53.
2. Gerber AL, *et al*. High expression of FOXP3 in primary melanoma is associated with tumor progression. Br J Dermatol. 2014 Jan; 170(1):103-9.
3. Ali HR, *et al*. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. Ann Oncol. 2014 Aug; 25(8):1536-43.
4. Liu H, *et al.* Tumor-infiltrating lymphocytes predict response to chemotherapy in patients with advance non-small cell lung cancer. Cancer Immunol Immunother. 2012 Oct; 61(10):1849-56.
5. Tao H, *et al.* Density of tumor-infiltrating FOXP3+ T cells as a response marker for induction chemoradiotherapy and a potential prognostic factor in patients treated with trimodality therapy for locally advanced non-small cell lung cancer. Ann Thorac Cardiovasc Surg. 2014; 20(6):980-6.
6. Ling A, *et al*. The intratumoural subsite and relation of CD8(+) and FOXP3(+) T lymphocytes in colorectal cancer provide important prognostic clues. Br J Cancer. 2014 May 13; 110(10):2551-9.
7. Frey DM, *et al.* High frequency of tumor-infiltrating FOXP3(+) regulatory T cells predicts improved survival in mismatch repair-proficient colorectal cancer patients. Int J Cancer. 2010 Jun 1; 126(11):2635-43.
8. Sloan S, *et al.* FOXP3+ regulatory T-cell counts correlate with histological response in Crohn's colitis treated with infliximab. Pathol Int. 2014 Dec; 64(12):624-7.
9. Stenard F, *et al*. Decreases in circulating CD4+CD25hiFOXP3+ cells and increases in intragraft FOXP3+ cells accompany allograft rejection in pediatric liver allograft recipients. Pediatr Transplant. 2009 Feb; 13(1):70-80.
10. 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."

References Cont'd:

11. 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
Lung Adenocarcinoma	0	4
Lung Squamous Carcinoma	0	1
Breast Cancer	0	5
Colon Cancer	0	5
Prostate Cancer	0	5
Bladder Cancer	0	5

Note: Tumor infiltrating lymphocytes are positive.

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