ROR gamma T

Concentrated and Prediluted Monoclonal Antibody
901-3208-072017

BIOCARE MEDICAL

Catalog Number:	**ACI 3208 A, B**	**API 3208 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

ROR gamma T [6F3.1] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of ROR gamma T 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:

Retineic-acid-receptor-related orphan nuclear receptor gamma (RORγt) is considered to be one of the master regulators in the development of T helper 17 cells (Th17 cells), which have an essential role in the development of many autoimmune disorders, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease and psoriasis (1). RORγT was initially identified as a transcription factor required for thymopoiesis by maintaining survival of $CD4^+CD8^+$ thymocytes. Therefore, RORγt is selectively expressed in the thymus and other immune system tissues although RORγt mRNA is detected in many tissues (2,3). Regulatory T cells can co-express RORγt and FOXP3 and are shown to be both pro-inflammatory and immunosuppressive (4). Studies have also shown a subset of $CD8^+RORγt^+$ T cells expressing a low level of PD-1 and a high level of OX40 were associated with reduced patient survival thus $CD8^+RORγt^+$ T cells are proinflammatory (5). RORγt seems to be a key regulator of immune homeostasis, proinflammatory, and may have potential for therapeutic targets in inflammatory diseases (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, 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: 6F3.1

Isotype: IgG2a/kappa

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

Epitope/Antigen: GST-tagged recombinant protein corresponding to human ROR gamma T

Cellular Localization: Nuclear

Positive Tissue Control: Small intestine (Peyer's patch), tonsil, thymus

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) (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

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Precautions Cont'd:

reagents and specimens. If reagents or specimens come into 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. Zhang Y, *et al*. ROR nuclear receptors: structures, related diseases, and drug discovery. Acta Pharmacol Sin. 2015; 36:71–87.
2. Guo Y, *et al*. Inhibition of RORγT Skewes TCR α Gene Rearrangement and Limits T Cell Repertoire Diversity. Cell Rep. 2016; 17:3206-3218.
3. Eberl G, *et al*. An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. Nat Immunol. 2004; 5:64–73.
4. Chellappa S, *et al*. Regulatory T cells that co-express RORγt and FOXP3 are pro-inflammatory and immunosuppressive and expand in human pancreatic cancer. Oncoimmunology. 2015; 29:5:e1102828.
5. Chellappa S, *et al*. CD8+ T Cells That Coexpress RORγt and T-bet Are Functionally Impaired and Expand in Patients with Distal Bile Duct Cancer. J Immunol. 2017; 198:1729-1739.
6. Ivanov II, *et al*. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 2006; 126:1121-1133.
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.

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

Tissue	Positive Cases	Total Cases
Breast Cancer	0	15
Colon Cancer	0	10
Lung Cancer	0	12
Prostate Cancer	0	15
Inflammatory Bowel Disease	5	5

Note: Tumor-infiltrating lymphocytes are stained.

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

Tissue	Positive Cases	Total Cases
Cerebrum	0	3
Cerebellum	0	3
Adrenal	0	3
Ovary	0	2
Pancreas	0	3
Parathyroid	0	1
Pituitary	0	2
Testis	0	3
Thyroid	0	3
Breast	0	3
Spleen	3	3
Tonsil	3	3
Thymus	3	3
Lung	0	3
Heart	0	2
Esophagus	0	1
Stomach	0	3
Small Intestine	0	3
Colon	0	3
Liver	0	3
Salivary Gland	0	2
Kidney	0	3
Prostate	0	2
Uterus	0	2
Cervix	0	2
Skeletal Muscle	0	3
Skin	0	3
Peripheral Nerve	0	3
Linging Cells	0	2

Note: Tissue lymphocytes are stained.

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