# GITR [CAL8]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3221-071318

BIOCARE MEDICAL

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|---|---|---|
| **Catalog Number:** | **ACI 3221 A, B** | **API 3221 AA** |
| **Description:** | 0.1, 0.5, ml concentrated | 6.0 ml, prediluted |
| **Dilution:** | 1:100 | Ready-to-use |
| **Diluent:** | Van Gogh Yellow | N/A |

**Intended Use:**
For In Vitro Diagnostic Use
GITR [CAL8] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of GITR 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:**
GITR (Glucocorticoid-induced TNF-R-related protein, TNFRSF18, CD357) is a co-stimulatory cell surface receptor constitutively expressed at high levels on T regulatory cells (Tregs), at intermediate levels on NK cells, and at low levels on naïve and memory T cells, and macrophage and dendritic cells (DCs) (1-2). Of all immune subsets studied, activated Tregs exhibit the highest level of GITR, an important distinction that becomes more apparent during the *in vivo* evaluation of GITR modulation (1-2). GITR ligand, GITRL (TNFSF18), is also a member of the TNF superfamily and is predominantly expressed by activated antigen presenting cells (APCs), including DCs, macrophage and activated B cells (2-4). The expression of both GITR and GITRL are not limited to hematopoietic cells. For example, GITR has been reported to be expressed at intermediate levels on epidermal keratinocytes and osteoclast precursors, whereas GITRL has been detected at high levels on endothelial cells, particularly following exposure to type I IFN (2). Therefore, tonsillar squamous epithelia would be the internal positive control for GITR and endothelial cells would be internal positive control for GITRL. The expression of GITR by tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment has been found to be higher than levels expressed by peripheral lymphocytes, indicating local T cell activation (5). Agonizing agents of this pathway have been considered as a way to increase the immune antitumor activity, although the clinical utility of such agents depends on the presence of T cells in the tumor and the subset of TILs which may vary among different malignancies (6). Thus, selection of patients who will derive the most benefit from this therapy is still unclear. Immune-related adverse events should also be considered. Preclinical data suggests that GITR therapy appears to be better tolerated than anti-CTLA4 agents (5-6). GITR modulation in the preclinical models has shown promising antitumor activity via significant increase in effector T cells and decrease in Tregs (5). Several human monoclonal antibodies that agonize GITR are currently undergoing phase I clinical studies in various solid malignancies. Preliminary results demonstrate an acceptable safety profile without dose limiting toxicities (7-9).

**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, 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; others not tested
**Clone:** CAL8
**Isotype:** IgG1
**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration.
**Epitope/Antigen:** Synthetic peptide derived from a region of the GITR 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 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 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.
**Technical Note:**
This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS 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

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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) (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. Shimizu J, *et al.* Stimulation of CD25+CD4+ regulatory T cells through GITR breaks immunological self-tolerance. Nature Immunol. 2002; 3:135-42.
2. Nocentini G, Riccardi C. GITR: a modulator of immune response and inflammation. Adv Exp Med Biol. 2009; 647:156-73.
3. Krausz LT, *et al.* GITR-GITRL system, a novel player in shock and inflammation. Scientific World Journal. 2007; 7:533-66.
4. Hanabuchi S, *et al.* Human plasmacytoid predendritic cells activate NK cells through glucocorticoid-induced tumor necrosis factor receptor-ligand (GITRL). Blood. 2006; 107:3617-23.
5. Dempke WCM, *et al.* Second- and third-generation drugs for immuno-oncology treatment-The more the better? Eur J Cancer. 2017; 74:55–72.
6. Knee DA, *et al.* Rationale for anti-GITR cancer immunotherapy. Eur J Cancer. 2016; 67:1–10.
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 were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

| Tissue | Positive Cases | Total Cases |
|---|---|---|
| Breast Cancer | 1 | 9 |
| Colon Cancer | 0 | 14 |
| Lung Cancer | 0 | 8 |
| Prostate Cancer | 0 | 15 |

Tumor-infiltrating lymphocytes (TILs) are positive in all cases when present.

**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 | 1 | 1 |
| Pituitary | 0 | 1 |
| Testis | 0 | 3 |
| Thyroid | 0 | 3 |
| Breast | 0 | 3 |
| Spleen | 3 | 3 |
| Tonsil | 10 | 10 |
| Thymus | 2 | 2 |
| Bone Marrow | 4 | 4 |
| Lung | 0 | 3 |
| Heart | 0 | 3 |
| Esophagus | 0 | 1 |
| Stomach | 0 | 3 |
| Small Intestine | 0 | 3 |
| Colon | 0 | 3 |
| Liver | 0 | 3 |
| Salivary Gland | 0 | 3 |
| Kidney | 0 | 3 |
| Prostate | 0 | 3 |
| Uterus | 0 | 2 |
| Cervix | 0 | 2 |
| Skeletal Muscle | 0 | 3 |
| Skin | 0 | 3 |
| Peripheral Nerve | 0 | 3 |
| Linging Cells | 0 | 3 |


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