

Anti-GITR

Catalog No.	Description
AR915-5RE	6 ml of Ready-to-Use Antibody for use with BioGenex Super Sensitive™ Detection Systems OR equivalent detection system
AR915-10RE	10 ml of Ready-to-Use Antibody in a barcode labeled vial for use with BioGenex Super Sensitive™ Detection Systems and i6000™ Automated Staining Systems
PU915-UPE	1 ml of Concentrated Antibody for use with BioGenex Super Sensitive™ Detection Systems OR equivalent detection system
PU915-5UPE	0.5 ml of Concentrated Antibody for use with BioGenex Super Sensitive™ Detection Systems OR equivalent detection system
AW915-YCDE	Ready-to-Use Antibody in Barcode labeled vial for use on the Xmatrx® Elite/Ultra Staining System, 160 tests
AW915-50DE	Ready-to-Use Antibody in Barcode labeled vial for use on the Xmatrx® Elite/Ultra Staining System, 50 tests

Clone	Species	Ig Class
Polyclonal	Rabbit	IgG

Intended Use

For In Vitro Diagnostic Use. This antibody is designed for the specific localization of GITR in formalin-fixed, paraffin-embedded (FFPE) tissue sections. Evaluation must be performed by a qualified pathologist.

Summary and Explanation

GITR (Glucocorticoid-induced TNF receptor family-regulated gene), also known as TNFRS18, belongs to the TNF receptor superfamily (TNFRS) (1). GITR is widely expressed in different cells of the immune system and its activation triggers the production of proinflammatory cytokines (2, 3). GITR is constitutively expressed at high levels on Tregs and at low levels on naive and memory T cells. Furthermore, activation of GITR with agonistic antibodies has been shown to amplify the antitumor immune responses in animal models by increasing the proliferation and function of effector T cells and possibly also by abrogating the suppressive function of Tregs cells.

Storage and Handling

Store at 2-8°C. Fresh dilutions, if required, should be prepared prior to use and are stable and steady for up to one day at room temperature (20-26°C). Diluted antibody preparations can be refrigerated or frozen for extended shelf life.

Principles of the Procedure

Antigen detection by immunohistochemistry (IHC) is a two-step process wherein the primary antibody binds to the antigen of interest and that binding is detected by a chromogen. The [primary antibody](#) may be used in IHC using manual techniques or BioGenex Automated Staining System. Positive and negative controls should always be run simultaneously with all patient specimens.

Reagents Provided

Rabbit Polyclonal Antibody GITR is affinity purified and diluted in PBS, pH 7.2, containing 1% BSA and 0.09% sodium azide.

Dilution of Primary Antibody

BioGenex Ready-to-Use antibodies have been optimized for use with the recommended BioGenex Detection System and should not require further dilution.

BioGenex concentrated antibodies must be diluted in accordance with the recommended protocol when used with the recommended BioGenex Detection System.

Recommended Protocol

Refer to the following table for conditions specifically recommended for this antibody. Refer to the BioGenex website for guidance on specific staining protocols or other requirements.

Parameter	BioGenex Recommendations
Control Tissue	Tonsil and Spleen as available with BioGenex FB-915PE* & FG-915PE*
Recommended Dilution for Concentrated Antibody	1:50-100 in HK941**
Recommended Pretreatment (Manual/i6000)**	EZ-AR2 (HK522-XAK)
Recommended Pretreatment (Xmatrx)	EZ-AR2 Elegance (HX032-YCD)
Antibody Incubation (Manual/i6000)	30 -60 min at RT
Antibody Incubation (Xmatrx)	30 -60 min at RT
Detection System for Manual, Xmatrx & i6000 systems***	Use BioGenex Two-Step OR One-Step Super Sensitive™ Polymer-HRP IHC Detection System/DAB; see p. 2 for more information

*FB: positive control barrier slides, FG: positive control non-barrier slides. Xmatrx requires barrier slides.

**Pretreatment times will vary based on individual microwave power.

***For automation systems (Xmatrx-Elite, Xmatrx-Ultra & i6000 Diagnostics), refer to the factory protocols provided with the instrument.

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Detection System	Two-Step HRP Kit	One-Step HRP Kit	Link and Label Kit
Manual	QD440-XAKE (1000 Test)	QD630-XAKE (1000 Test)	QP300-XAKE (1000 Test)
	QD430-XAKE (1000 Test)		
	QD420-YIKE (500 Test)	QD620-XAKE (500 Test)	QP900-9LE (500 Test)
	QD400-60KE (60 Test)		
Xmatrix - Automation	QD550-YCDE (200 Test)	QD610-YADE (200 Test)	N/A
i6000 - Automation	QD410-YAXE (200 Test)	QD610-YAXE (200 Test)	N/A
For more information, visit www.biogenex.com .			

Precautions

This product contains sodium azide at concentrations of less than 0.1%. Sodium azide is not classified as a hazardous chemical at the product concentrations, but proper handling protocols should be observed. For more information, a Safety Data Sheet (SDS) for sodium azide is available upon request. Dispose of unused reagents according to Local, State and Federal Regulations. Wear suitable Personal Protective Equipment, do not pipette reagents by mouth, and avoid contact of reagents and specimens with skin and mucous membranes. If reagents or specimens come in contact with sensitive area, wash with copious amounts of water.

Quality Control

Refer to BioGenex detection system documents for guidance on general quality control procedures.

Troubleshooting

Refer to the troubleshooting section in the documentation for BioGenex Detection Systems (or equivalent detection systems) for remedial actions on detection system related issues, or contact BioGenex Technical Support Department at 1-800-421-4149 or support@biogenex.com or your local distributor to report unusual staining.

Expected Results

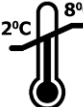
This antibody stains secreted and cell membrane in positive cells in formalin-fixed, paraffin embedded tissue sections. An example image of a tissue section stained with this antibody can be found on the product page on the BioGenex website. Interpretation of the staining result is solely the responsibility of the user. Experimental results should be confirmed by a medically-established diagnostic product or procedure.

Limitations of the Procedure

Improper tissue handling and processing prior to immunostaining can lead to inconsistent results. Variations in embedding and fixation or the nature of the tissue may lead to variations in results. Endogenous peroxidase activity or pseudo peroxidase activity in erythrocytes and tissue biotin may result in non-specific staining based on the detection system employed. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive with horseradish peroxidase systems. Improper counterstaining and mounting may compromise the interpretation of results.

Bibliography

1. Kanamaru F, et al. Costimulation via glucocorticoid-induced TNF receptor in both conventional and CD25+ regulatory CD4+ T cells. J Immunol. 172 (12) 7306–14 (2004).
2. Bae E, et al. Glucocorticoid-induced tumour necrosis factor receptor-related protein-mediated macrophage stimulation may induce cellular adhesion and cytokine expression in rheumatoid arthritis. Clin Exp Immunol. 148 (3) 410–8 (2007).
3. Kim WJ, et al. Glucocorticoid-induced tumour necrosis factor receptor family related protein (GITR) mediates inflammatory activation of macrophages that can destabilize atherosclerotic plaques. Immunology. 119 (3) 421–9 (2006).
4. Stephens GL, et al. Engagement of glucocorticoid-induced TNFR family-related receptor on effector T cells by its ligand mediates resistance to suppression by CD4+CD25+ T cells. J Immunol. 173 (8) 5008–20 (2004).
5. Knee DA, et al. Rationale for anti-GITR cancer immunotherapy. European Journal of Cancer. 67 1-10 (2016).

	Temperature Limitation		In Vitro Diagnostic Medical Device
	Use By Date		Batch Code
	Non-Sterile		Consult Instructions for Use
	Representative in the European Community		Manufacturer

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