

# Anti- CTLA-4 [F-8]

Catalog No.	Description		
AMC20-5M	6 ml of Ready-to-Use Antibody for use with BioGenex Super Sensitive <sup>TM</sup> Detection Systems OR equivalent detection system		
AMC20-10M	10 ml of Ready-to-Use Antibody in a barcode labeled vial for use with BioGenex Super Sensitive <sup>TM</sup> Detection Systems and i6000 <sup>TM</sup> Automated Staining Systems		
MUC20-UC	1 ml of Concentrated Antibody for use with BioGenex Super Sensitive <sup>TM</sup> Detection Systems OR equivalent detection system		
MUC20-5UC	0.5 ml of Concentrated Antibody for use with BioGenex Super Sensitive <sup>TM</sup> Detection Systems OR equivalent detection system		
AXC20-YCD Ready-to-Use Antibody in Barcode labeled vial for use on the Xmatrx® Elite/Ultra Staining System, 160 tests			
AXC20-50D	Ready-to-Use Antibody in Barcode labeled vial for use on the Xmatrx <sup>®</sup> Elite/Ultra Staining System, 50 tests		

Clone	Species	Ig Class
F-8	Mouse	IgG1

#### **Intended Use**

For In Vitro Diagnostic Use. This antibody is designed for the specific localization of CTLA-4 in formalin-fixed, paraffinembedded (FFPE) tissue sections. Evaluation must be performed by a qualified pathologist.

# **Summary and Explanation**

CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4) is composed of a single Ig V-like extracellular domain, a transmembrane domain and an intracellular domain and belongs to immunoglobulin (Ig) gene superfamily. It is structurally homologous to CD28 and is expressed on CD4+ cytotoxic T cells. CTLA-4 is a strong inhibitor of T-cell activation and is functions in T-cell apoptosis. CTLA-4 is also found intracellularly in regulatory T-cells and is important for their function.

#### **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.

# Emergo Europe, Prinsessegracht 20, 2514AP The Hague, The Netherlands

# **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**

Mouse Monoclonal Antibody CTLA-4 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	Heart tissue as available with Biogenex FB-C20M* & FG-C20M*	
Recommended Dilution for Concentrated Antibody	1:10-25 in HK941	
Recommended Pretreatment (Manual/i6000)**	EZ-AR2 (HK522-XAK) / EZ-AR2 Elegance (HK547- 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 25°C	
Detection System for Manual, Xmatrx & i6000 systems***	Use BioGenex Two-Step <b>OR</b> One-Step Super Sensitive <sup>™</sup> Polymer-HRP IHC Detection System/DAB; see p. 2 for more information	

<sup>\*</sup>FB: positive control micro chamber slides, FG: positive control slides. Xmatrx requires micro chamber microscopic slides.

<sup>\*\*</sup>Pretreatment times will vary based on individual microwave power.

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

Detection System	Two-Step HRP Kit	One-Step HRP Kit	Link and Label Kit
Manual	QD440-XAKEN (1000 Test) QD430-XAKEN (1000 Test)	QD630-XAKEN (1000 Test)	QP300-XAKE (1000 Test)
ivianuai	QD420-YIKEN (500 Test) QD400-60KEN (60 Test)	QD620-XAKEN (500 Test)	QP900-9LE (500 Test)
Xmatrx - 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 membrane and cytoplasm 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. Freeman, G.J., et al. 1991. Structure, expression, and T cell costimulatoryactivity of the murine homologue of the human B lymphocyte activationantigen B7. J. Exp. Med. 174: 625-631.
- Schwartz, R.H. 1992. Costimulation of T lymphocytes: the role of CD28,CTLA-4, and B7/BB1 in Interleukin-2 production and immunotherapy. Cell71: 1065-1068.

2°€ 8°€	Temperature Limitation	IVD	In Vitro Diagnostic Medical Device
$\boxtimes$	Use By Date	LOT	Batch Code
NON STERILE	Non-Sterile	[]i	Consult Instructions for Use
EC REP	Representative in the European Community		Manufacturer

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