

## TNF-alpha (Tumor Necrosis Factor alpha)

Mouse Monoclonal Antibody

Mob502

PDM230

PDM230-10MM

**\*\*This data sheet is applicable to all sizes (volume) of product.**

Actual volume is indicated on vial.

Document #: DS-0544-C

Effective Date: 12/18/2023

Immunogen	Clone	Species	Isotype	Primary Antibody Diluent
Recombinant full-length human TNF-alpha protein	DBM15.28	Mouse	IgM, kappa	K004

*Lot specific Ig concentration available upon request.*

Catalog #	Description
Mob502	Concentrated antibody for use with Diagnostic BioSystems PolyVue™ Plus - Two Step Detection System
PDM230	Ready to use antibody for use with Diagnostic BioSystems PolyVue™ Plus - Two Step Detection System
PDM230-10MM	Ready to use antibody for use with Diagnostic BioSystems Montage PolyVue Plus™ Auto Detection System & Montage™ 360 System

### Intended Use

For In Vitro Diagnostic Use. This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections, to be viewed by light microscopy. Clinical interpretation of staining results should be accompanied by histological studies with proper controls. Patients' clinical histories and other relevant diagnostic tests should be utilized by a qualified person(s) when evaluating and interpreting results.

### Summary and Explanation

This MAb recognizes human 17-26kDa protein, which is identified as cytokine TNF- $\alpha$  (Tumor Necrosis Factor-alpha). Monomeric human TNF- $\alpha$  is a 157 amino acid protein (non-glycosylated) with a reported molecular weight of 17 kDa and can be expressed as a free molecule, also TNF- $\alpha$  is generated as a precursor form called transmembrane TNF- $\alpha$  can be expressed as a cell surface type II polypeptide consisting of 233 amino acid residues molecular weight 26 kDa. TNF- $\alpha$  is an important cell-signaling component of the immune system. It is a protein secreted by LPS stimulated macrophages, and causes tumor necrosis when injected into tumor bearing mice. TNF- $\alpha$  is currently being evaluated in treatment of certain cancers and AIDS Related Complex.

### Format

This product is supplied as purified immunoglobulin and contains sodium azide as a preservative.

### Principles of the Procedures

Antigen detection by immunohistochemistry (IHC) is a two-step process involving first, the binding of a primary antibody to the antigen of interest, and second, the detection of bound antibody by a chromogen. The primary

antibody may be used in IHC using manual techniques or using Diagnostic BioSystems Automated Montage 360™ Staining System.

### Dilution of Primary Antibody

Diagnostic BioSystems ready to use antibodies have been optimized for use with the recommended Diagnostic BioSystems Detection System and do not require further dilution. Further dilution may result in loss of sensitivity. The user must validate any such change.

Diagnostic BioSystems concentrated antibodies must be diluted in accordance with the staining procedure when used with the recommended Diagnostic BioSystems detection system. Use of any detection methods other than the recommended systems and protocols require validation by the user. Antibody dilutions should be appropriately adjusted and verified according to the detection system used.

### Materials Required But Not Provided

Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at [www.dbiosys.com](http://www.dbiosys.com)

### Storage and Handling

Store at 2-8°C. This antibody is suitable for use until the expiration date when stored at 2-8°C. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly. Unused portions of antibody preparation should be discarded after one day.

The presence of precipitate or an unusual odor indicates that the antibody is deteriorating and should not be used.

Positive and negative controls should be run simultaneously with all patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or [techsupport@dbiosys.com](mailto:techsupport@dbiosys.com).

### Specimen Collection and Preparation

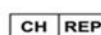
Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981; Sheehan & Hrapchak, 1980) for further details on specimen preparation.

The user is advised to validate the use of the products with their tissue specimens prepared and handled in accordance with their laboratory practices.

### Precautions

This product is a single-use, non-sterile, in vitro diagnostic device.

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<sub>3</sub>) 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). 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. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other



than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

#### Treatment of Tissues Prior to Staining

Place the slides in the recommended antigen retrieval solution using Diagnostic BioSystems Montage Opus™ Antigen Retrieval System. Allow slides to cool down for 20 minutes prior to staining.

#### Staining Procedure

Refer to the following table for conditions specifically recommended for this antibody. Refer to the Diagnostic BioSystems PolyVue™ Plus—Two Step Detection System or Montage PolyVue Plus™ Auto Detection System for guidance on specific staining protocols or other requirements.

Parameter	Diagnostic BioSystems Recommendations
Positive Control	Histiocytoma, Colon, Pancreas
Concentrated Dilution	1:50-1:200
Pretreatment	EDTA Buffer pH 8.0
Incubation Time & Temperature	30 min @ RT
Detection System	PolyVue™ Plus - Two Step Detection System or Montage PolyVue Plus™ Auto Detection System for Montage 360 System
Tissue Type	FFPE

#### 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.

#### Troubleshooting

Contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2, techsupport@dbiosys.com or your local distributor to report unusual staining.

#### Cellular Localization

Cytoplasmic and extracellular (secreted)

#### Limitations of the Procedure

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can also cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue may cause variations in results (Nadji and Morales, 1983). Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. Tissues containing Hepatitis B surface Antigen (HBsAg) may give a false positive with horseradish peroxidase systems (Omata et al, 1980). Improper counterstaining and mounting may compromise the interpretation of results.

#### Performance Characteristics

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, and 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 Diagnostic BioSystems products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These

products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

#### References

- I) Bradley JR. TNF-mediated inflammatory disease. *J Pathol* 2008;214:149-60.
- II) Pennica D, *et al.* Human tumor necrosis factor: precursor structure, cDNA cloning, expression, and homology to lymphotoxin. *Nature* 1984;312:724-9.
- III) Shealy DJ, *et al.* Anti-TNF antibodies: lessons from the past, roadmap for the future. *Handb Exp Pharmacol.* 2008;(181):101-29.
- IV) Carswell EA, *et al.* An endotoxin-induced serum factor that causes necrosis of tumors. *PNAS (USA)* 1975, **72**:3666–3670.
- V) Bebok Z; *et al.* Prognostic relevance of transforming growth factor alpha (TGF-alpha) and tumor necrosis factor alpha (TNF-alpha) detected in breast cancer tissues by immunohistochemistry. *Breast Cancer Research and Treatment*, 1994, 29(3):229-35.

