

## TNF-alpha (Clone: TNF1172) Mouse Monoclonal Antibody

<b>PRODUCT INFORMATION:</b>	<b>PERFORMANCE CHARACTERISTICS:</b>
<b>PM214</b> 6ml Ready to use	<b>Localization:</b> Cytoplasm & membrane
<b>PM214</b> 3ml Ready to use	<b>Retrieval Buffer:</b> Tris-EDTA, pH 9.0
<b>CM214</b> 1ml Concentrated	<b>Incubation:</b> 60 minutes
<b>CM214</b> 0.5ml Concentrated	<b>Positive control:</b> Histiocytoma
<b>CM214</b> 0.1ml Concentrated	
<b>HAM214</b> 6ml Ready to use	
<b>HAM214</b> 3ml Ready to use	

### INTENDED USE

#### For *in vitro* diagnostic use only

This antibody is intended for use in qualitatively identify TNF-alpha antigen by light microscopy in formalin fixed, paraffin embedded (FFPE) tissue sections using immunohistochemical (IHC) detection methodology. Interpretation of any positive or negative staining must be complemented with the evaluation of proper known controls (Positive and Negative) and must be made within the context of the patient's clinical history and other diagnostic tests. A qualified and trained pathologist must perform evaluation of the test. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains

### SUMMARY AND EXPLANATION

This MAb recognizes human 17-26kDa protein, which is identified as cytokine TNF-alpha (Tumor Necrosis Factor-alpha). TNF-alpha can be expressed as a 17kDa free molecule, or as a 26kDa membrane protein. TNF-alpha is a protein secreted by lipopolysaccharide-stimulated macrophages, and causes tumor necrosis when injected into tumor bearing mice. TNF alpha causes cytolysis of certain transformed cells, being synergistic with interferon gamma in its cytotoxicity. Although it has little effect on many cultured normal human cells, TNF alpha appears to be directly toxic to vascular endothelial cells. Other actions of TNF alpha include stimulating growth of human fibroblasts and other cell lines, activating polymorphonuclear neutrophils and osteoclasts, and induction of interleukin 1, prostaglandin E2 and collagenase production. TNF alpha is currently being evaluated in treatment of certain cancers and AIDS Related Complex.

### PRINCIPLE OF THE PROCEDURE

The identification of the antigen on the FFPE tissues is carried out using the above stated antibody. The antigen and antibody complex is visualized using an enzyme coupled (HRP/AP) secondary antibody with specific binding to the primary antibody, this complex is visualized by the enzymatic activation of the chromogen resulting to a visible reaction production of the antigenic site. Each and every step involves precise time and optimal temperature and the results are interpreted using a light microscope by a qualified and trained pathologist.

### REAGENT PROVIDED

**Concentrated format:** Antibody to TNF-alpha is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% of sodium azide (NaN<sub>3</sub>).

**Recommended dilutions:** 1:50 – 1:100

The antibody dilution and protocol may vary depending on the specimen preparation and specific application. Optimal conditions should be determined by individual laboratory.

**Pre-diluted format:** PathnSitu's ready to use antibodies are pre-titrated to optimal staining conditions. Further dilution will affect the efficacy of the antibody and may yield to sub-optimal staining.

**Immunogen:** Recombinant full length human TNF alpha.

**Host, Isotype:** Mouse, IgM/kappa

### STORAGE AND HANDLING

**Storage Recommendations:** Store at 2-8°C. When stored at the appropriate conditions, the antibody is stable until expiry. Do not use the antibody after expiration date provided on the vial in any condition.

To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the vial into the refrigerated conditions in an upright position. The contents of the vial should be used within 9 months from the

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opening of the vial.

### SPECIMEN PREPARATION

#### Staining Recommendations:

Routinely processed, FFPE tissues are suitable for use with this primary antibody, when used PathnSitu's Poly Excel HRP/DAB detection system. The recommended tissue fixative is 10% neutral buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification. Thickness of the sections should be 2-5µm. Slides should be stained once the sections are made as antigenicity of the cut sections may diminish over a period of time. It is recommended to stain known positive and negative controls simultaneously with unknown specimens.

### PRECAUTIONS

1. This product should be used by qualified and trained professional users only
2. The product contains < 0.1% of sodium azide as preservative and is not classified hazardous, refer MSDS for further details
3. As with any product derived from biological sources, proper handling procedures should be used
4. Do not use reagents after expiration date
5. Use protective clothing and gloves, while handling reagents
6. All hazardous materials should be disposed according to local state and federal regulations
7. Avoid microbial contamination of reagents as it may lead to incorrect results

### STAINING PROCEDURE

**Antigen Retrieval Solution:** Use **Tris-EDTA Buffer (Cat#PS009)** as antigen retrieval solution.

**Heat Retrieval Method:** Retrieve sections under steam pressure for 20 minutes using PathnSitu's MERS (Multi Epitope Retrieval System) for optimal retrieval of the epitopes, allow solution to cool at the room temperature, transfer the tissue sections/slides to the distilled water prior to the primary antibody application.

**Primary Antibody:** Cover the tissue sections with primary antibody and incubate for 60 min at room temperature when used PathnSitu's PolyExcel Detection System.

**Detection System:** Refer to PathnSitu's PolyExcel HRP/ DAB detection system protocol for optimal staining results.

### QUALITY CONTROL

The recommended positive tissue control for TNF-alpha is Histiocytoma. A positive and negative tissue control must be run with every staining procedure performed for monitoring the correct performance of processed tissue and test reagents. A negative tissue controls provide an indication of non-specific background staining. If the results are not expected in positive and negative controls the test must be considered invalid and entire procedure must be cross verified. Individual laboratory must establish their own quality control to validate the process and antibody when opened a vial.

### INTERPRETATION OF RESULTS

TNF-alpha stains the Cytoplasm and Membrane. A qualified experienced/trained pathologist must interpret the results in the patient's sample along with the positive and negative controls.

### PERFORMANCE CHARACTERISTICS

PathnSitu products will undergo a thorough quality control check before it is released to the market. The antibody showed consistent specific and sensitive staining on the multiple positive tissue controls tested, by inter run, intra run and lot based studies. The antibody is stable for the expiry mentioned on the labels which is determined by real time or accelerated methods.

### TROUBLESHOOTING

1. Follow the antibody specific protocol recommendations according to data sheet provided
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, antibody freezing and thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping or inaccurate results
3. Do not allow the section to dry out during the entire IHC process

4. Excessive or incomplete counterstaining may compromise the interpretation of the results
5. If unusual results occur, contact PathnSitu's Technical Support at +91-40-2701 5544 or E-mail: [techsupport@pathnsitu.com](mailto:techsupport@pathnsitu.com)

#### LIMITATIONS AND WARRANTY

Authorized and skilled/trained personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified trained pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

#### BIBLIOGRAPHY

1. Bebok Z; Markus B; Nemeth P. 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.

#### EXPLANATION OF SYMBOLS

LOT- Lot number / Batch number



- Expiry

IVD

*In vitro* diagnostic use



Storage limitation