PathnSitu Biotechnologies

HIF-1 Alpha (Clone: EP118)
Rabbit Monoclonal Antibody

PRODUCT INFORMATION:

REF

MR1306	6ml Ready to use	
MR1306	3ml Ready to use	
MRC1306	1ml Concentrated	
MRC1306	0.5ml Concentrated	
MRC1306	0.1ml Concentrated	
MRH1306	6ml Ready to use	
MRH1306	3ml Ready to use	

PERFORMANCE CHARACTERISTICS:

Localization: Nucleus/ Cytoplasm
Retrieval Buffer: Tris-EDTA, pH 9.0
Incubation: 60 minutes
Positive control: Congested Spleen

INTENDED USE

For research use only
This antibody is intended for use in qualitatively identify HIF-1 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.

SUMMARY AND EXPLANATION

Hypoxia-inducible factor 1-alpha, also known as HIF-1-alpha, is a protein that in humans is encoded by the HIF1A gene. Two alternative transcripts encoding different isoforms have been identified, the alpha and beta subunits. HIF-1 plays an essential role in cellular and systemic responses to hypoxia.
HIF-1 is a critical mediator of the hypoxic response that upregulates expression of proteins that promote angiogenesis, anaerobic metabolism, and many other survival pathways. HIF-1alpha is expressed in many types of tumors. Overexpressed in the majority of common human cancers and their metastases, due to the presence of intratumoral hypoxia and as a result of mutations in genes encoding oncoproteins and tumor suppressors. Under normoxic conditions HIF-1 alpha has a short half-life. It is largely undetectable in cells or tissues grown under normoxic conditions. It is stabilized only at O2 concentrations below 5% and upon stabilization under hypoxic conditions HIF-1 translocates to the nucleus. The expression of HIF 1alpha is correlated with tumor angiogenesis,cancer progression and clinical outcome in various solid tumors including breast cancer, type 1 endometrial carcinoma, sarcoma, head and neck tumor and brain tumor. HIF-1 alpha may be of value in analyzing the cancer cell response to therapy.

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 a 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 HIF-1 Alpha is affinity purified and diluted in antibody diluent with 1% bovine serum albumin (BSA) and 0.05% of sodium azide (NaN3).
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-tittered to optimal staining conditions. Further dilution will affect the efficacy of the antibody and may yield to sub-optimal staining.
Immunogen: A synthetic peptide corresponding to residues near the C-terminus of human HIF-1 Alpha protein
Host, Isotype: Rabbit, IgG

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.

DS-MR1306-B.

To ensure proper regent 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 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 controls for HIF-1 alpha are Congested spleen and Inflammatory tissue (Overexpressed in the majority of common human cancers and their metastases, due to the presence of intratumoral hypoxia) 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

HIF-1 alpha stains the Nucleus or Cytoplasm. 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

RUO

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
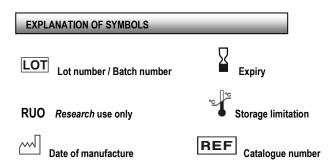
LIMITATIONS AND WARRANTY

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. Iyer NV. Et al: Genomics 1998, 52:159-165
2. Wang GL,et, al:Proc Natl acad Sci USA 1995, 92:5510-5514
3. Carmeliet P, et al :Nature 1998, 394:485-490
4. Sutter CH, et al: Proc Natl Acad Sci USA 2000, 97:4748-4753
5. Talks KL, et al: Am J Pathol 2000, 157: 411-421
6. Bos R, et al : J Natl Cancer Inst2001, 93:309-314
7. Pansare V, et al : Mod Pathol 2007, 20:35-43
8. Brizel DM, et al: Cancer Res 1996, 56:941-943
9. Zagzag D, et al: Cancer 2000, 88:2606-2618
10. Vaupel P, et al :Semin Oncol 2001, 28:29-35

HIF-1 alpha, EP118 antibody has been created by Epitomics Inc., using Epitomics proprietary rabbit monoclonal antibody technology covered under Patent No.'s 5,675,063 and 7,402,409.

EXPLANATION OF SYMBOLS

LOT **Lot number / Batch number**

RUO *Research* **use only**

Date of manufacture

Expiry

Storage limitation

REF **Catalogue number**