

# HIF-1 alpha

Concentrated Rabbit Monoclonal Antibody  
901-349-113017

**BIOCARE**  
M E D I C A L

**Catalog Number:** CME 349 A, B  
**Description:** 0.1, 0.5 ml, concentrated  
**Dilution:** 1:100  
**Diluent:** Da Vinci Green

## Intended Use:

For In Vitro Diagnostic Use

HIF-1 alpha [EP1215Y] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of HIF-1 alpha protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

## Summary and Explanation:

HIF-1 alpha has been shown to upregulate several genes to promote survival in hypoxic environments (1-3). Some of the important genes that are upregulated are erythropoietin, nitric oxide synthase (NOS), heme oxygenase 1 (HO-1), glucose transporters, and vascular growth factors necessary for the maintenance of homeostasis in low oxygen conditions. Oxygen-breathing species express this highly-conserved transcriptional complex (3). Studies have shown the HIF-1 alpha expression also plays a role in cancer (1-2). There is evidence that tumor hypoxia promotes metastasis through the induction of MET overexpression by HIF-1 alpha. The mechanism of tumor hypoxia promoting metastasis remains uncertain. HIF-1 alpha is a key mediator of the cellular response to hypoxia and binds the MET promoter, resulting in increased expression of MET (1-3). Studies have shown that in breast cancer, MET overexpression is associated with metastatic disease and poor prognosis.

## Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, an enzyme labeled polymer is added to bind to the primary antibody. This detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Rabbit monoclonal

**Species Reactivity:** Human

**Clone:** EP1215Y

**Isotype:** IgG

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration.

**Epitope/Antigen:** HIF-1 alpha

**Cellular Localization:** Nuclear

**Positive Tissue Control:** Breast cancer

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

## Storage and Stability:

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

## Protocol Recommendations:

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1.

**Pretreatment Solution (recommended):** Diva

## Pretreatment Protocol:

Heat Retrieval Method:

Preheat the retrieval solution to 95°C for 30 minutes and then place slides in the preheated solution if using Biocare's Decloaking Chamber Pro or Decloaking Chamber Plus. If using Biocare's Decloaking Chamber NxGen, place slides into the retrieval solution without preheating. Retrieve at 95°C for 40 minutes. Allow solution to cool for 20 minutes and then wash in distilled water.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

**Primary Antibody:** Incubate for 30 minutes at RT.

**Probe:** N/A

**Polymer:** Incubate for 30 minutes at RT with a secondary-conjugated polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Biocare's Warp Red.

## Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

## Technical Note:

This antibody has been standardized with Biocare's MACH 2 detection system. Use TBS buffer for washing steps.

## Limitations:

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, 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 Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

## 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

## Precautions:

1. 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) (4)

2. 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

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## Precautions Cont'd:

and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (4)

3. Microbial contamination of reagents may result in an increase in nonspecific staining.

4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.

5. Do not use reagent after the expiration date printed on the vial.

6. The SDS is available upon request and is located at <http://biocare.net>.

## Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

## References:

1. Takahashi Y, Nishikawa M, Takakura Y. Inhibition of tumor cell growth in the liver by RNA interference-mediated suppression of HIF-1 alpha expression in tumor cells and hepatocytes. *Gene Ther.* 2008 Apr; 15(8):572-82.

2. Jung SN, *et al.* Reactive oxygen species stabilize hypoxia-inducible factor-1 alpha protein and stimulate transcriptional activity via AMP-activated protein kinase in DU145 human prostate cancer cells. *Carcinogenesis.* 2008 Apr; 29(4):713-21.

3. Zur Nedden S, Tomaselli B, Baier-Bitterlich G. HIF-1 alpha is an essential effector for purine nucleoside-mediated neuroprotection against hypoxia in PC12 cells and primary cerebellar granule neurons. *J Neurochem.* 2008 Jun; 105(5):1901-14.

4. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."

5. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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