abcam

ab255733

Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25]

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR3664(2)-25
Purification technique	Affinity purification Protein A
Concentration	0.404 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/250
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Not recommended

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/400
Notes	-

Target data

See full target information P4HTM

Function Catalyzes the post-translational formation of 4-hydroxyproline in hypoxia-inducible factor (HIF) alpha proteins. Hydroxylates HIF1A at 'Pro-402' and 'Pro-564'. May function as a cellular oxygen sensor and, under normoxic conditions, may target

HIF through the hydroxylation for proteasomal degradation via the von Hippel-Lindau ubiquitination complex.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	HIF prolyl hydroxylases also known as EglN or prolyl hydroxylase domain proteins (PHDs) are enzymes that modulate the stability of hypoxia-inducible factors (HIFs). These enzymes primarily facilitate the hydroxylation of prolyl residues on HIF-α subunits which marks them for degradation via the ubiquitin-proteasome pathway. Mammals express three isoforms: PHD1 PHD2 and PHD3 with PHD2 being the most widely studied. PHDs are expressed ubiquitously with higher levels observed in the liver kidneys and lung. The molecular mass of these enzymes is typically around 40 kDa.

Biological function summary	HIF prolyl hydroxylases play an essential role in oxygen sensing and homeostasis by regulating the activity of HIF-α subunits. Under normoxic conditions the hydroxylation of HIF-α by these hydroxylases permits recognition by the von Hippel-Lindau (VHL) protein leading to proteasomal degradation. In hypoxic conditions the decreased activity of HIF prolyl hydroxylases allows HIF-α stabilization and heterodimerization with HIF-β facilitating transcription of target genes involved in angiogenesis and metabolism. PHD2 is predominantly responsible for targeting HIF-1α.
Pathways	HIF prolyl hydroxylases integrate into the hypoxia response pathway. Oxygen-dependent regulation of HIF by these hydroxylases affects processes such as angiogenesis and erythropoiesis through interaction with several proteins including endothelial growth factor (VEGF) and erythropoietin (EPO). Beyond the hypoxia pathway they participate in metabolic pathways influencing cellular energy homeostasis thereby impacting proteins involved in metabolic regulation.
Associated diseases and disorders	HIF prolyl hydroxylases associate with cancer and ischemic diseases. Dysregulation of these hydroxylases can lead to excessive stabilization of HIF-α which promotes tumor growth and metastasis due to enhanced angiogenesis and altered metabolism associated with cancer cells. In ischemic conditions reduced prolyl hydroxylases activity can offer protection by inducing adaptive responses through HIF-α stabilization. Their connectivity to the VHL protein further links them to von Hippel-Lindau disease characterized by tumor development in multiple organs.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

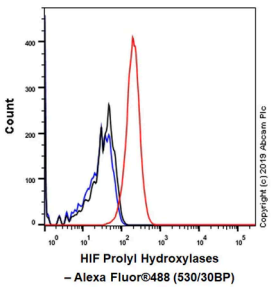
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:

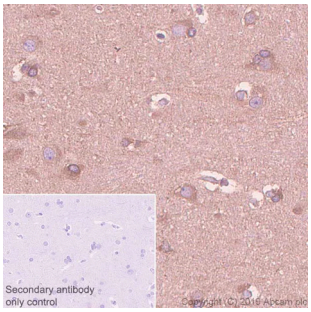
5 product images



Flow Cytometry (Intracellular) - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] (ab255733)

HIF Prolyl Hydroxylases Flow Cytometry (Intracellular) staining using rabbit Anti-HIF Prolyl Hydroxylases antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293 (human embryonic kidney epithelial cell) cells labelling HIF Prolyl Hydroxylases with ab255733 at 1/400 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).
Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

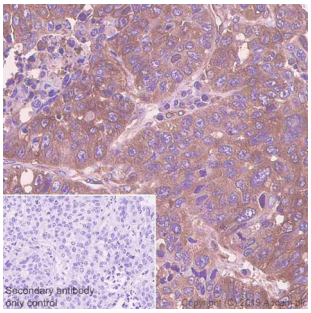


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] (ab255733)

HIF Prolyl Hydroxylases Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HIF Prolyl Hydroxylases antibody

Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling HIF Prolyl Hydroxylases with ab255733 at 1/250 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining on human cerebrum (PMID: 17726031, 12163023). The section was incubated with ab255733 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).



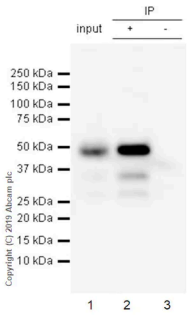
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] (ab255733)

HIF Prolyl Hydroxylases Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HIF Prolyl Hydroxylases antibody

Immunohistochemical analysis of paraffin-embedded Human lung cancer tissue labeling HIF Prolyl Hydroxylases with ab255733 at 1/250 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining on human lung cancer (PMID: 24382781). The section was incubated with ab255733 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).



Immunoprecipitation - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] (ab255733)

HIF Prolyl Hydroxylases was immunoprecipitated from 0.35 mg HEK-293 (human embryonic kidney epithelial cell) whole cell lysate 10µg with ab255733 at 1/30 dilution. Western blot was performed on the immunoprecipitate using ab255733 1/1000 dilution (0.4 µg/ml). VeriBlot for IP Detection Reagent (HRP) (ab131366) was used as the secondary antibody at 1/5000 dilution.
Lane 1: HEK-293 (human embryonic kidney epithelial cell) whole cell lysate 10µg

Lane 2: ab255733 IP in HEK-293 whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab255733 in HEK-293 whole cell lysate.

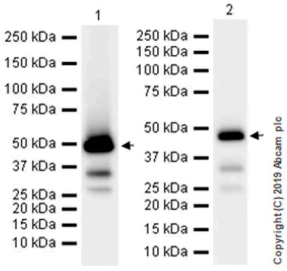
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

All lanes:
Immunoprecipitation - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] (ab255733)

Predicted band size: 57 kDa

Observed band size: 47 kDa



Western blot - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] (ab255733)

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.
All lanes:
Western blot - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] (ab255733) at 1/1000 dilution

Lane 1:
HEK-293 (human embryonic kidney epithelial cell) whole cell lysate at 10 µg

Lane 2:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 57 kDa

Observed band size: 47 kDa

Exposure time: 20s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.