

Product datasheet

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

Recombinant

RabMAb

6 Images

Overview

Product name	Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25]
Description	Rabbit monoclonal [EPR3664(2)-25] to HIF Prolyl Hydroxylases
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), WB, IHC-P, IP Unsuitable for: ICC/IF
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human cerebrum and lung cancer tissue Flow Cyt (intra): HEK-293 cells IP: HEK-293 whole cell lysate WB: HEK-293 and HeLa whole cell lysates
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal

Clone number EPR3664(2)-25

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255733 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/400.
WB		1/1000. Detects a band of approximately 47 kDa (predicted molecular weight: 57 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/30.

Application notes Is unsuitable for ICC/IF.

Target

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.

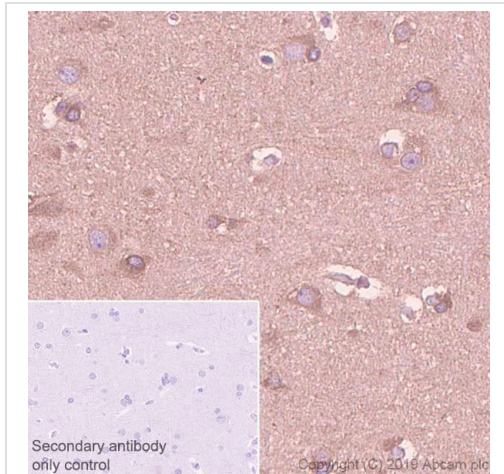
Tissue specificity Widely expressed with highest levels in adult pancreas, heart, skeletal muscle, brain, placenta, kidney and adrenal gland. Expressed at lower levels in epiphyseal cartilage and in fibroblasts.

Sequence similarities Contains 2 EF-hand domains.
Contains 1 Fe2OG dioxygenase domain.

Post-translational modifications Glycosylated.

Cellular localization Endoplasmic reticulum membrane.

Images

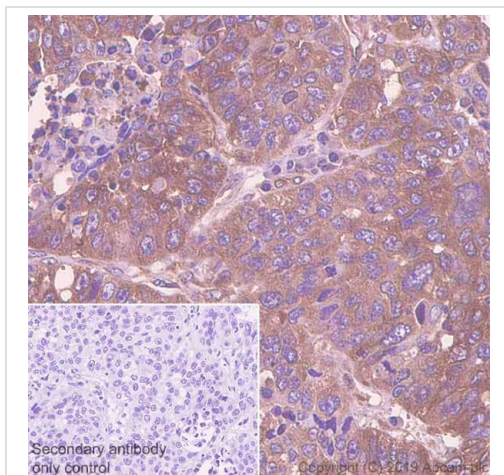


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] (ab255733)

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**).

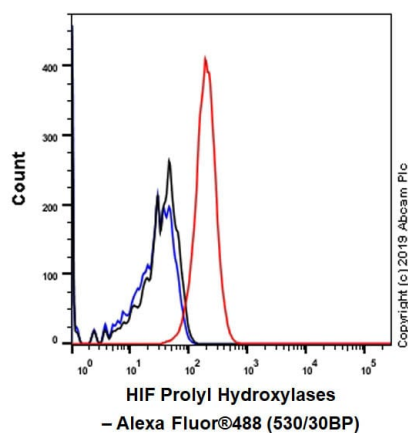


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] (ab255733)

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**).



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

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.



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.

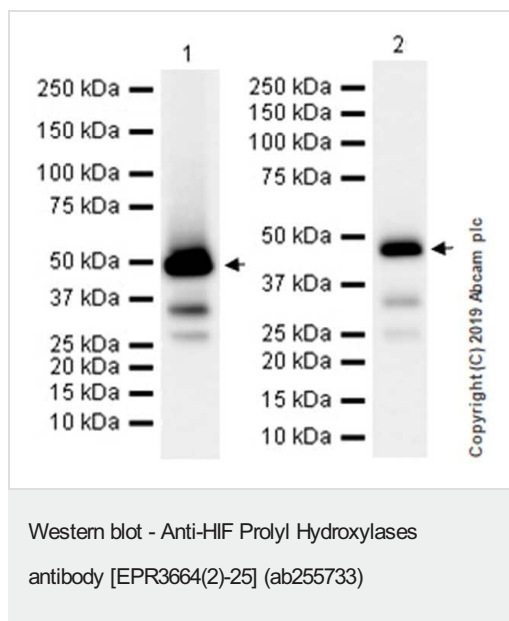
Lane 1: HEK-293 (human embryonic kidney epithelial cell) whole cell lysate 10µg

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 : 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

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

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 57 kDa

Observed band size: 47 kDa

Exposure time: 20 seconds

Blocking/Diluting buffer and concentration: 5% NFDm/TBST.

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

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

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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