

Product datasheet

Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] - BSA and Azide free ab256316

Recombinant RabMAb

5 Images

Overview

Product name	Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] - BSA and Azide free
Description	Rabbit monoclonal [EPR3664(2)-25] to HIF Prolyl Hydroxylases - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: IHC-P, IP, Flow Cyt (Intra), WB 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>ab256316 is the carrier-free version of ab255733.</p> <p>Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.</p> <p>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.</p> <p>Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.</p> <p>This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.</p> <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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3664(2)-25
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab256316 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

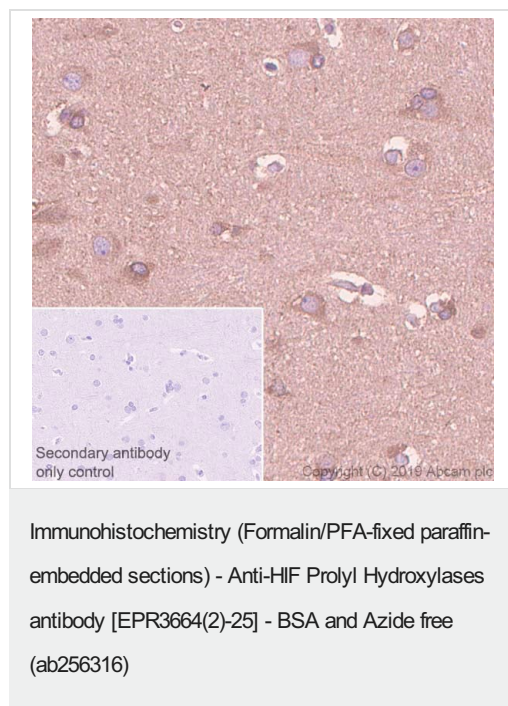
Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 47 kDa (predicted molecular weight: 57 kDa).

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.

Images

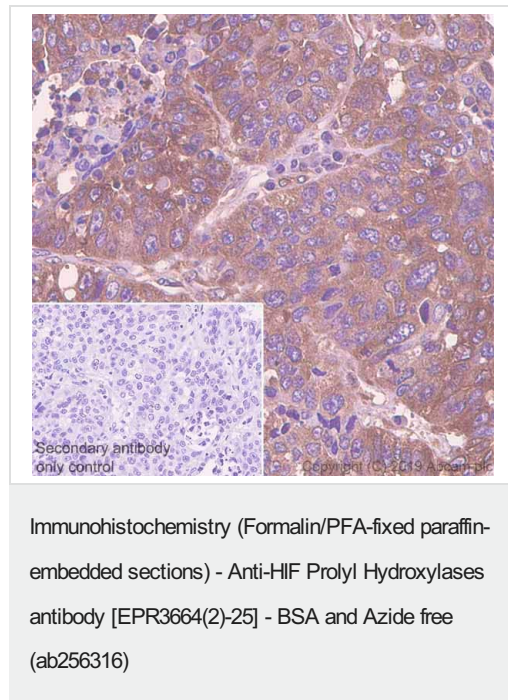


Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling HIF Prolyl Hydroxylases with [ab255733](#) at 1/250 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)). Cytoplasmic staining on human cerebrum (PMID: 17726031, 12163023). 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](#)).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab255733](#)).

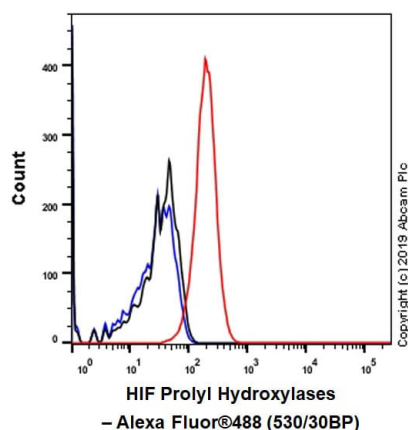


Immunohistochemical analysis of paraffin-embedded Human lung cancer tissue labeling HIF Prolyl Hydroxylases with [ab255733](#) at 1/250 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)). Cytoplasmic staining on human lung cancer (PMID: 24382781). The 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](#)).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab255733](#)).



Flow Cytometry (Intracellular) - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] - BSA and Azide free (ab256316)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab255733**).



Immunoprecipitation - Anti-HIF Prolyl Hydroxylases antibody [EPR3664(2)-25] - BSA and Azide free (ab256316)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab255733**).

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

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

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