

HIF1A Recombinant Polyclonal Antibody (16HCLC)

Catalog Number710059

Product data sheet

Details		Species Reactivity	
Size	100 µg	Species reactivity	Human
Host/Isotope	Rabbit / IgG	Tested Applications	
Class	Recombinant Polyclonal	ChIP assay (ChIP)	3 µg
Type	Antibody	ELISA (ELISA)	Assay-Dependent
Clone	16HCLC	Western Blot (WB)	0.5-1 µg/mL
Immunogen	proprietary	Immunocytochemistry (ICC/IF)	1 µg/mL
Conjugate	Unconjugated	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Form	Liquid		
Concentration	0.5 mg/mL		
Purification	Protein A		
Storage buffer	PBS		
Contains	0.09% sodium azide		
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.		

Product specific information

This antibody is predicted to react with mouse based on sequence homology. Recombinant rabbit polyclonal antibodies are unique offerings from Thermo Fisher Scientific. They are comprised of a selection of multiple different recombinant monoclonal antibodies, providing the best of both worlds - the sensitivity of polyclonal antibodies with the specificity of monoclonal antibodies - all delivered with the consistency only found in a recombinant antibody. While functionally the same as a polyclonal antibody - recognizing multiple epitope sites on the target and producing higher detection sensitivity for low abundance targets - a recombinant rabbit polyclonal antibody has a known mixture of light and heavy chains. The exact population can be produced in every lot, circumventing the biological variability typically associated with polyclonal antibody production.

Background/Target Information

HIF1- α (HIF1A) is a subunit of HIF1, which is a transcription factor found in mammalian cells cultured under reduced oxygen tension. HIF-1 is a heterodimer consisting of an α and β subunit, both belonging to the basic-helix-loop-helix Per-aryl hydrocarbon receptor nuclear translocator-Sim (PAS) family of transcription factors. HIF1 functions as a transcriptional regulator of the adaptive response to hypoxia. Under hypoxic conditions, HIF-1 activates the transcription of over 40 genes, including erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, HILPDA, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. HIF1- α regulates hypoxia-mediated apoptosis, cell proliferation and tumor angiogenesis. Hypoxia which induces p53 protein accumulation, directly interacts with HIF1- α and reduces hypoxia-induced expression of HIF1- α by promoting MDM2-mediated ubiquitination and proteasomal degradation under hypoxic conditions. Recent studies suggest that induction of NOX4 by HIF1- α contributes to maintain ROS levels after hypoxia and hypoxia-induced proliferation. In humans, it is located on the q arm of chromosome 14. The C-terminal of HIF1A binds to p300. p300/CBP-HIF complexes participate in the induction of hypoxia-responsive genes, including VEGF. Hypoxia contributes significantly to the pathophysiology of major categories of human disease, including myocardial and cerebral ischemia, cancer, pulmonary hypertension, congenital heart disease and chronic obstructive pulmonary disease.

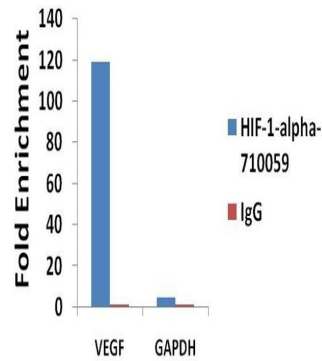
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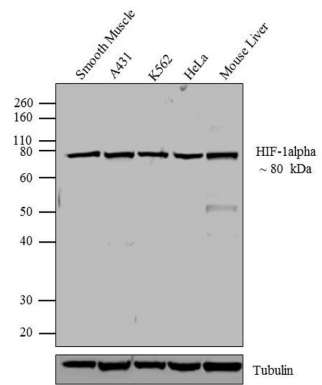
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Product Images For HIF1A Recombinant Polyclonal Antibody (16HCLC)



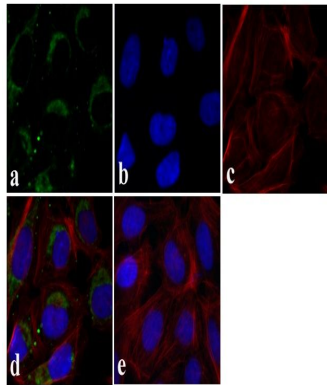
HIF1A Antibody (710059)

Antibody specificity was demonstrated through detection of enrichment of the target protein at specific gene loci. Chromatin Immunoprecipitation (ChIP) was performed using HIF1-alpha Recombinant Rabbit Polyclonal Antibody (Product # 710059) with relevant positive and negative target genes/ binding sites. {RE}



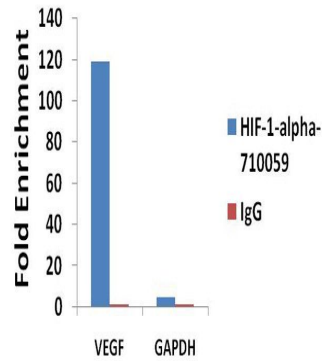
HIF1A Antibody (710059) in WB

Western blot analysis of HIF1A was performed by loading 20 µg of A431 (lane 1), K562 (lane 2), HeLa (lane 3) and mouse liver (lane 4) cell lysates using Novex®NuPAGE®4-12 % Bis-Tris gel (Product # NP0321BOX), XCell SureLock Electrophoresis System (Product # EI0002), Novex® Sharp Pre-Stained Protein Standard (Product # LC5800), and iBlot® Dry Blotting System (Product # IB21001). Proteins were transferred to a nitrocellulose membrane and blocked with 5 % skim milk for 1 hour at room temperature. HIF1A was detected at ~80 kDa using HIF1A Recombinant Rabbit Polyclonal Antibody (Product # 710059) at 0.5-1 µg/mL in 2.5 % skim milk at 4°C overnight on a rocking platform. Goat anti-Rabbit IgG-HRP Secondary Antibody (Product # G-21234) at 1:5000 dilution was used and chemiluminescent detection was performed using Pierce™ ECL Western blotting Substrate (Product # 32106).



HIF1A Antibody (710059) in ICC/IF

Immunofluorescent analysis of HIF1-alpha was done on 70% confluent log phase U-2 OS cells. The cells were fixed with 4% paraformaldehyde for 15 minutes, and blocked with 5% BSA for 1 hour at room temperature. The cells were labeled with HIF1-alpha Recombinant Rabbit Polyclonal Antibody (Product # 710059) at 1 µg/mL in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor 488 Goat anti-Rabbit IgG Secondary Antibody (Product # A-11008) at a dilution of 1:400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor 594 Phalloidin (Product # A12381). Panel d is a merged image showing cytoplasmic localization and panel e is a no primary antibody control. The images were captured at 20X magnification.



HIF1A Antibody (710059) in ChIP

ChIP- qPCR analysis of HIF1-alpha was performed with 3 µg/mL of the HIF1-alpha Recombinant Rabbit Polyclonal Antibody (Product # 710059) on sheared chromatin from 2 million HeLa cells serum starved overnight and serum released for 1h using the MAGnify Chromatin Immunoprecipitation System (Product # 49-2024). Normal rabbit IgG (3 µg/mL) was used as a negative IP control. The purified DNA from each ChIP sample was analyzed by StepOnePlus Real-Time PCR System (Product # 4376600) with primers for the promoter of active VEGF gene, used as positive control targets, and the GAPDH gene, used as negative control target. Data is presented as fold enrichment of the antibody signal versus the negative control IgG using the comparative CT method.

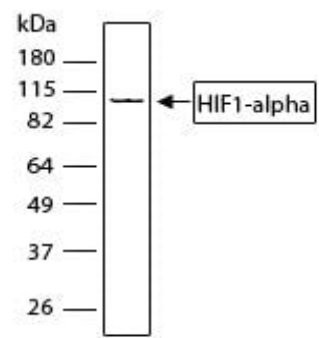
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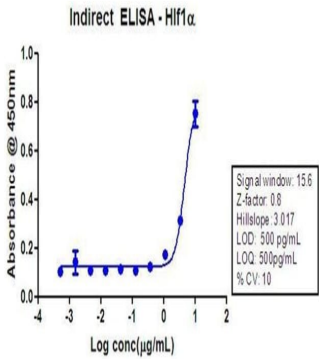
HIF1A Antibody (710059) in WB

Western blot analysis of HIF-1 alpha in whole cell extract from HEK293 cells (30 µg per lane) using a HIF-1 alpha Recombinant Rabbit Polyclonal Antibody (Product # 710059) at a dilution of 1 µg/mL. Detection was performed using an HRP-conjugated Goat anti-Rabbit secondary antibody at a dilution of 1:5000 followed by chemiluminescence (ECL). Results show a band at ~93 kDa.



HIF1A Antibody (710059) in ELISA

Indirect ELISA analysis of HIF-1 alpha in HEK293 cell lysate (300 ng/well) using a HIF-1 alpha Recombinant Rabbit Polyclonal Antibody (Product # 710059) and TMB (Product # SB01) as a substrate.



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