PRODUCT INFORMATION

Product name：HIF3A antibody

Product type： Primary antibodies

Description： Rabbit polyclonal to HIF3A

Immunogen：3 synthetic peptides (human) conjugated to KLH

Reacts with： Human, Mouse

Tested applications： ELISA, WB & IF

GENE INFORMATION

Gene Symbol：HIF3A

Gene Name：hypoxia inducible factor 3, alpha subunit

Ensembl ID：ENSG00000124440

Entrez GeneID：64344

GenBank Accession number：AK027725

Omim ID：609976

Swiss-Prot：Q9Y2N7

Molecular weight of HIF3A： 72.4kDa

Function：Involved in adaptive response to hypoxia. Suppresses hypoxia-inducible expression of HIF1A and EPAS1. Binds to core DNA sequence 5'-TACGTG-3' within the hypoxia response element (HRE) of target gene promoters. The complex HIF3A-ARNT activates the transcription of reporter genes driven by HRE. Isoform 4 has a dominant-negative function of inactivating HIF1A-mediated transcription. Isoform 4 attenuates the binding of HIF1A to hypoxia-responsive elements (HRE), thus inhibiting HRE-driven transcription. Hypoxia induces down-regulation of isoform 4, leading to activation of HIF1A in hypoxia. Conversely, upon restoring normoxia, the expression of isoform 4 increases and thereby secure an inhibition of HIF1A activity. Isoform 4 may be a negative regulator of hypoxia-inducible gene expression in the kidney and may be involved in renal tumorigenesis. Functions as an inhibitor of angiogenesis in the cornea

Expected subcellular localization：Nucleus. Cytoplasm.

Note: In the nuclei of all periportal and perivenous hepatocytes. In the distal perivenous zone, detected in the cytoplasm of the hepatocytes

Summary：The protein encoded by this gene is the alpha-3 subunit of one of several alpha/beta-subunit heterodimeric transcription factors that regulate many adaptive responses to low oxygen tension (hypoxia). The alpha-3 subunit lacks the transactivation domain found in factors containing either the alpha-1 or alpha-2 subunits. It is thought that factors containing the alpha-3 subunit are negative regulators of hypoxia-inducible gene expression. Multiple alternatively spliced transcript variants have been found for this gene. [provided by RefSeq, Mar 2011]

APPLICATION NOTE

Recommended dilution：

- **ELISA: Antibody specificity was verified by direct ELISA against the 3 immunogen peptides. A minimum titer of 1/60000 is determined. Appropriate specificity controls were run.**
- **WB (recombinant protein): 1/5000.**
- **WB (cell lysate): 1/500.**
- **IF: 1:500**

Optimal dilutions/concentration should be determined by the end user.

Raised in： Rabbit

Clonality： Polyclonal

Isotype： IgG

Purity： Purified Antibody

Storage buffer： 0.5 X PBS, 50% glycerol containing a final concentration of 0.1% BSA and 0.01% Thimerosal.

Form：Liquid

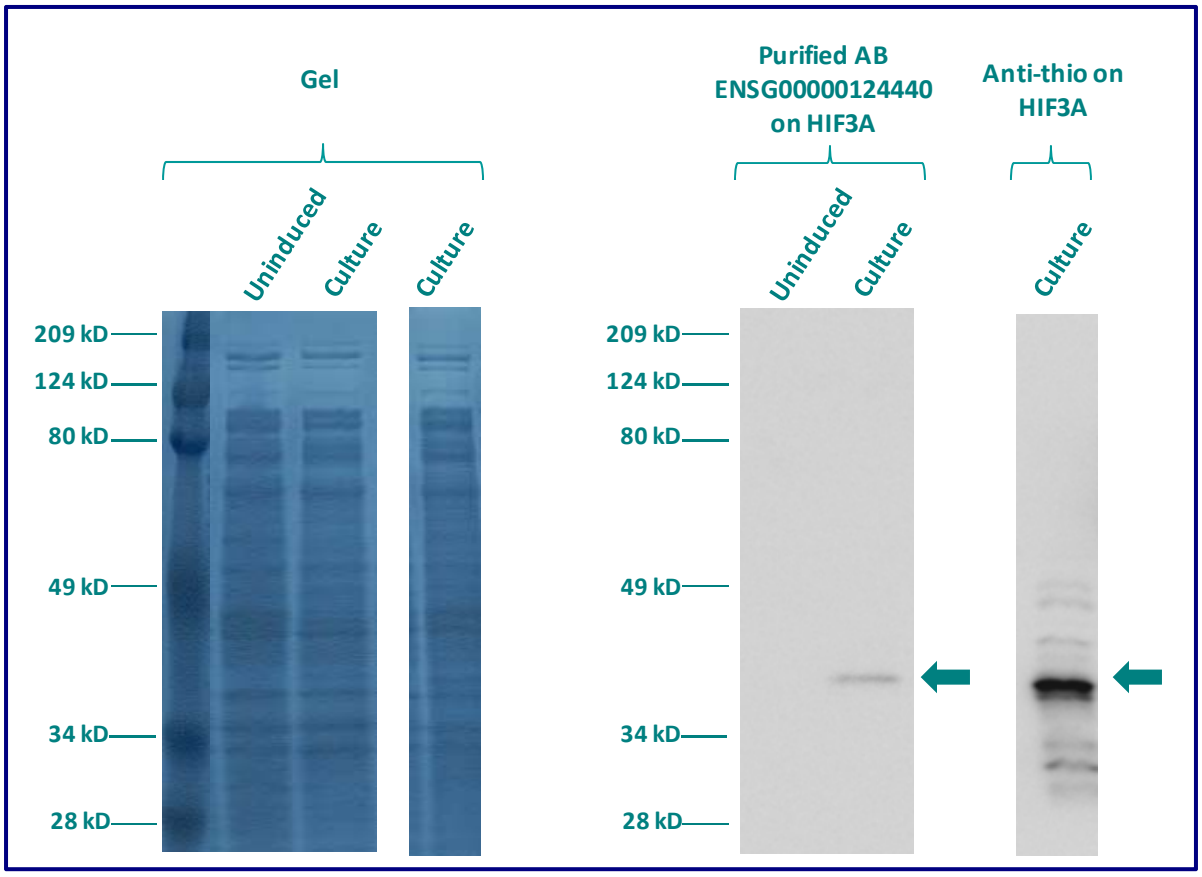
Storage instruction： Store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

WESTERN BLOT ON RECOMBINANT PROTEIN

The purified antibody ENSG00000124440 has been tested at 1/5000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells).

An anti-thio has been tested at 1/5000 on induced culture of E.coli (one shot Top10 competent cells) as a positive control.

Plasmid name： pBAD-DEST49.
Molecular weight of HIF3A：86.4kDa (72.4kDa + another 14kDa for the tag).



Gel concentration: 10%
Blocking: in 5% non-fat milk-PBST solution
1st Antibody: The antibodies are diluted in blocking buffer.

- **Dilute the purified antibody ENSG00000124440 at 1: 5000**
- **Dilute the anti-thio at 1:5000**

60 minutes of incubation
2nd Antibody: The antibody is diluted in blocking buffer.

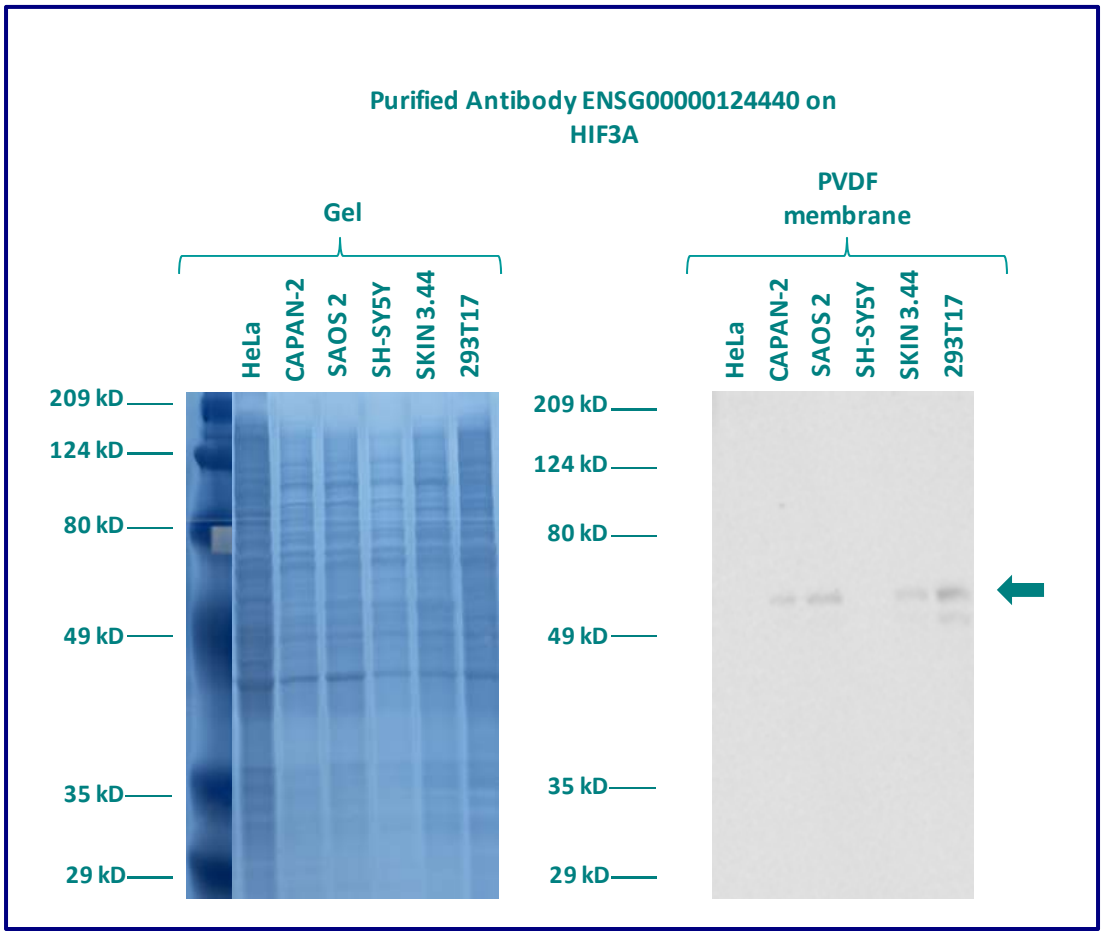
- **Dilute the anti-Rabbit IgG HRP conjugated at 1/10000**

60 minutes of incubation

WESTERN BLOT ANALYSIS ON CELL LYSATES

The purified antibody ENSG00000124440 has been tested at a concentration of 1/500 on total protein extract of various cell lines (HeLa, CAPAN-2, SAOS 2, SH-SY5Y, SKIN 3.44 & 293T17).

Molecular weight of HIF3A isoforms : 72.4, 69.9, 68.9, 66.1 & 39.9kDa



Gel concentration: 10%
Blocking: in 5% non-fat milk-PBST solution
1st Antibody: The antibodies are diluted in blocking buffer.

- **Dilute the purified antibody ENSG00000124440 at 1: 500**

60 minutes of incubation
2nd Antibody: The antibody is diluted in blocking buffer.

- **Dilute the anti-Rabbit IgG HRP conjugated at 1/10000**

60 minutes of incubation

IMMUNOFLUORESCENCE ANALYSIS

Immunofluorescence analysis of Hypoxia-inducible factor 3-alpha (HIF3A) expression in 3 cells lines (HELA, Capan-2, Skin 3,44). The purified Antibody ENSG00000124440 has been tested at 1/500.

Red staining : cytoskeleton (microtubules/α-tubuline)

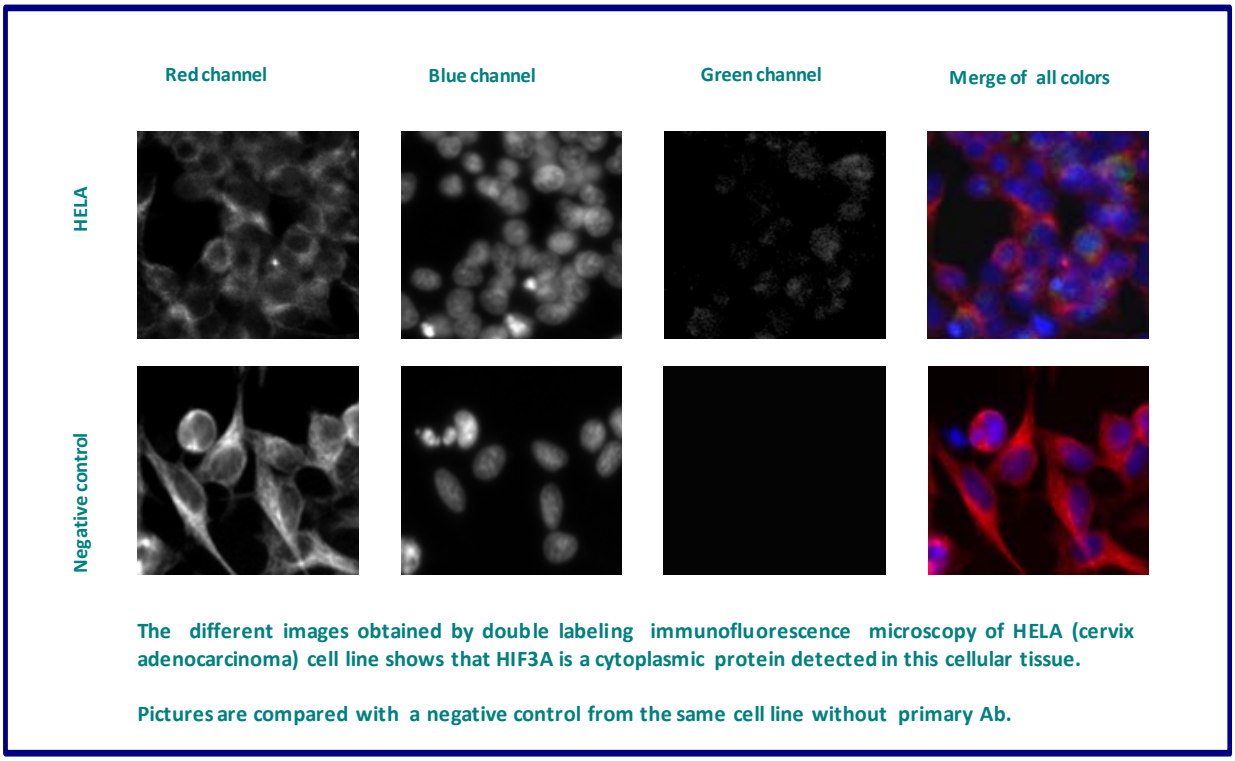
Blue staining : nucleus (Hoechst)

Green staining : anti- HIF3A antibody (purified)

Expected subcellular location : Nucleus. Cytoplasm.

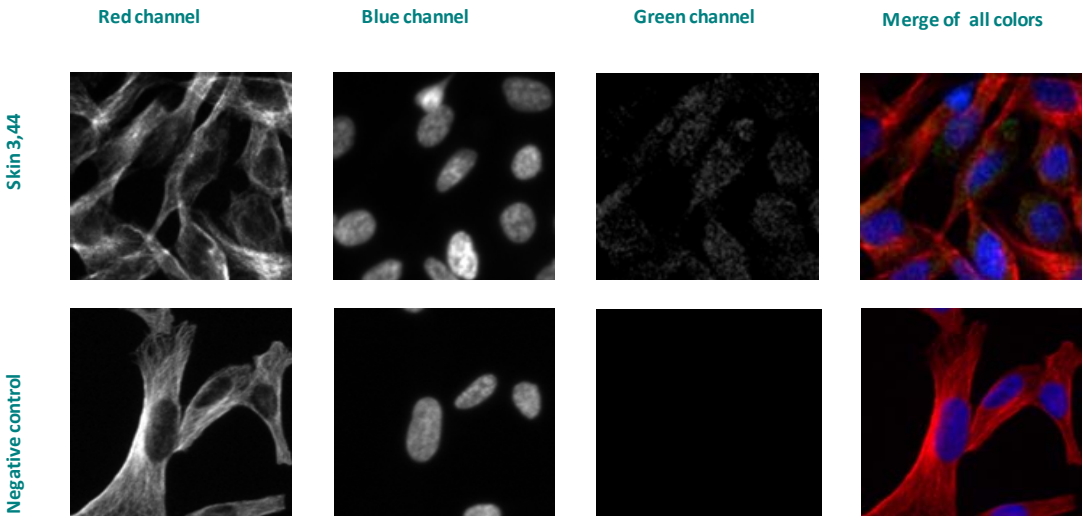
Note: In the nuclei of all periportal and perivenous hepatocytes. In the distal perivenous zone, detected in the cytoplasm of the hepatocytes

Expected tissue specificity : Expressed in kidney. Expressed abundantly in lung epithelial cells. Expression is regulated in an oxygen-dependent manner



The different images obtained by double labeling immunofluorescence microscopy of HELA (cervix adenocarcinoma) cell line shows that HIF3A is a cytoplasmic protein detected in this cellular tissue.

Pictures are compared with a negative control from the same cell line without primary Ab.



The different images obtained by double labeling immunofluorescence microscopy of Skin 3,44 (melanoma) cell line shows that HIF3A is a nuclear and cytoplasmic protein detected in this cellular tissue.

Pictures are compared with a negative control from the same cell line without primary Ab.

Remaining cell lines tested gave a positive result with a cytoplasmic distribution.