Product Datasheet

ARNT/HIF-1 beta Antibody
NB100-110

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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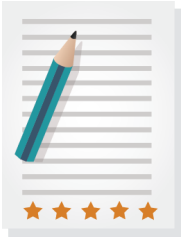
Updated 10/23/2024 v.20.1



NB100-110

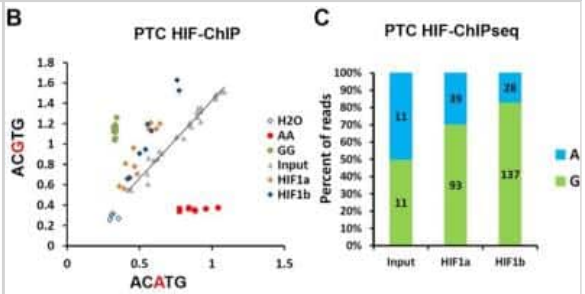
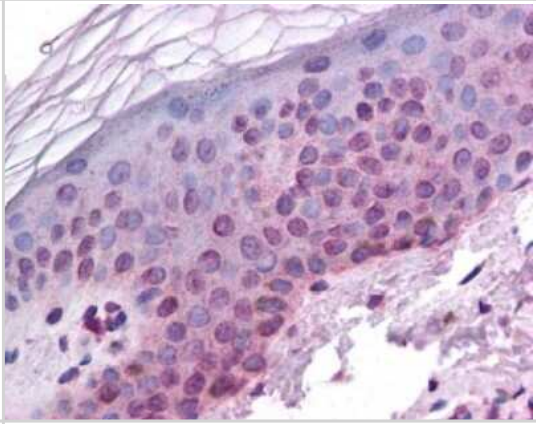
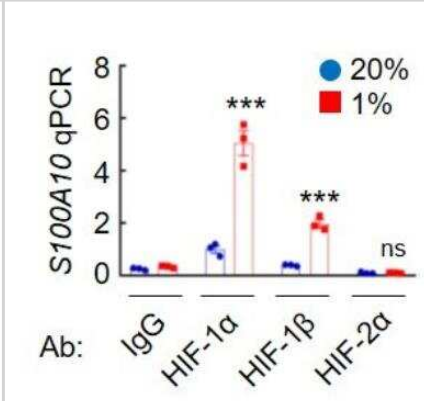
ARNT/HIF-1 beta Antibody

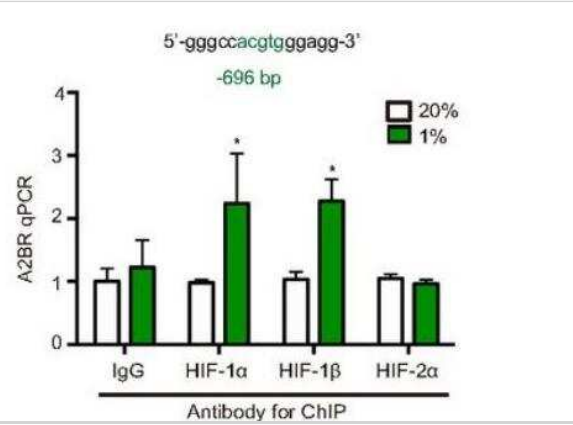
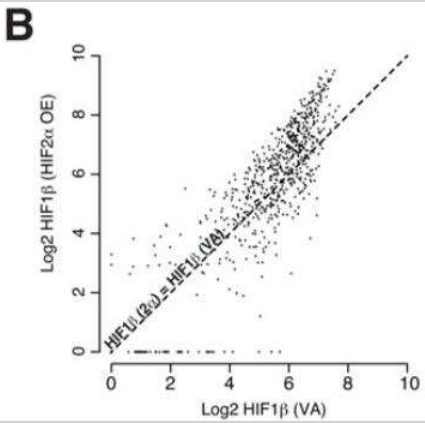
Product Information	
Unit Size	0.1 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Unpurified
Buffer	Whole antisera
Target Molecular Weight	86.6 kDa

Product Description	
Host	Rabbit
Gene ID	405
Gene Symbol	ARNT
Species	Human, Mouse, Rat, Bovine, Canine, Fish, Ferret, Sheep
Reactivity Notes	ARNT/HIF-1 beta antibody shows Fish reactivity as reported in scientific literature (PMID: 30334616). Canine reactivity reported in scientific literature (PMID:32885302)
Specificity/Sensitivity	ARNT/HIF-1 beta Antibody is specific for HIF-1 beta/ARNT. It is not known if NB100-110 cross-reacts with ARNT2 which is related to HIF-1 beta/ARNT but is the product of a different gene.
Immunogen	ARNT/HIF-1 beta Antibody was developed against a fusion protein to human HIF-1 beta containing amino acids 496-789. [UniProt# P27540]

Product Application Details	
Applications	Western Blot, Simple Western, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Gel Supershift Assay
Recommended Dilutions	Western Blot 1:2000, Simple Western 1:1000, Immunohistochemistry 1:150, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:150, Immunoblotting, Gel Supershift Assay, Chromatin Immunoprecipitation (ChIP) 1:10-1:500

Images

Chromatin Immunoprecipitation: ARNT/HIF-1 beta Antibody [NB100-110] - ChIP analysis with ARNT/HIF-1 beta antibody [NB100-110]. Allele specific HIF-binding at the rs12814794 associated enhancer. B) Allele-specific qPCR for rs12814794 from DNA fragments isolated in HIF-ChIP experiments (HIF-1A and HIF-1B) or input DNA from primary renal tubular cells (PTC, n = 4 individuals). DNA from individuals homozygous for the AA or GG genotype were used as positive controls. C) Quantification of the two different alleles (A or G) at rs12814794 in the ChIP-seq reads from HIF-1A and HIF-1B immunoprecipitations (PTC #1 Fig 3). Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pgen.1006872) licensed under a CC-BY license.	
Western Blot: ARNT/HIF-1 beta Antibody [NB100-110] - Analysis of HIF-1 beta using ARNT/HIF-1 beta antibody [NB100-110] in MCF7 whole cell lysate. Theoretical molecular weight is 86.6 kDa. Observed molecular weight ~95 kDa.	
Immunohistochemistry: ARNT/HIF-1 beta Antibody [NB100-110] - Immunohistochemical staining of human skin epidermis with ARNT/HIF-1 beta antibody [NB100-110].	
Chromatin Immunoprecipitation: ARNT/HIF-1 beta Antibody [NB100-110] - ChIP analysis with ARNT/HIF-1 beta antibody [NB100-110]. MDA-MB-231 cells were exposed to 20% or 1% O2 for 16 hours, and chromatin immunoprecipitation (ChIP) was performed with the indicated antibody (Ab). Primers flanking the HIF binding site were used for qPCR. ChIP image submitted by a verified customer review.	

Chromatin Immunoprecipitation (ChIP): ARNT/HIF-1 beta Antibody [NB100-110] - ChIP analysis using ARNT/HIF-1 beta antibody [NB100-110]. MCF7 were exposed to 20% or 1% O2 for 16h, and ChIP assays were performed using IgG or antibodies against HIF-1a, HIF-2a, HIF-1b. ChIP image submitted by a verified customer review.	
Chromatin Immunoprecipitation: ARNT/HIF-1 beta Antibody [NB100-110] - ChIP analysis with ARNT/HIF-1 beta antibody [NB100-110]. A scatter plot showing HIF-1B ChIP-seq signal at the same set of binding sites exhibiting a comparable increase in HIF-1B binding in cells expressing transfected HIF-2A. Image collected and cropped by CiteAb from the following publication (//dx.plos.org/10.1371/journal.pone.0134645) licensed under a CC-BY license.	
Simple Western: ARNT/HIF-1 beta Antibody [NB100-110] - Simple Western analysis using ARNT/HIF-1 beta antibody [NB100-110]. Lane view shows a specific band for HIF-1 beta in 0.5 mg/ml of Hypoxic HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system. Theoretical molecular weight is 86.6 kDa. Observed molecular weight ~106 kDa.	

Publications

Haiquan Lu, Yajing Lyu, Linh Tran, Jie Lan, Yangyiran Xie, Yongkang Yang, Naveena L Murugan, Yueyang J Wang, Gregg L Semenza HIF-1 recruits NANOG as a coactivator for TERT gene transcription in hypoxic breast cancer stem cells. Cell reports 2022-02-10 [PMID: 34592152]

Zuo Q, Yang Y, Lyu Y, Yang C, Chen C, Salman S, Huang T, Wicks E, Jackson W, Datan E, Qin W, Semenza G Plexin-B3 expression stimulates MET signaling, breast cancer stem cell specification, and lung metastasis. Cell Rep, 2023-02-28;42(3):112164. 2023-02-28 [PMID: 36857181]

Lyu Y, Yang Y, Talwar V et al Hypoxia-inducible factor 1 recruits FACT and RNF20/40 to mediate histone ubiquitination and transcriptional activation of target genes Cell Rep 2024-03-22 [PMID: 38517892] (ChIP, Human)

Grampp S, Kraus A, Skoczynski K et al. Hypoxia induces polycystin-1 expression in the renal epithelium Royal Society Open Science 2023-05-17 [PMID: 37206967]

Lombardi O, Li R, Halim S et al. Pan-cancer analysis of tissue and single-cell HIF-pathway activation using a conserved gene signature Cell Reports 2022-11-15 [PMID: 36384128] (Western Blot)

Wierenga ATJ, Cunningham A, Erdem A et al. HIF1/2-exerted control over glycolytic gene expression is not functionally relevant for glycolysis in human leukemic stem/progenitor cells Cancer & Metabolism 2019-12-27 [PMID: 31890203]

Orlando IMC, Lafleur VN, Storti F et al. Distal and proximal hypoxia response elements cooperate to regulate organ-specific erythropoietin gene expression Haematologica 2019-12-19 [PMID: 33256376]

Naas S, Krüger R, Knaup KX et al. Hypoxia controls expression of kidney-pathogenic MUC1 variants Life science alliance 2023-09-01 [PMID: 37316299] (ChIP, Human)

Grampp S, Krüger R, Lauer V et al. Hypoxia hits APOL1 in the kidney Kidney international 2023-04-23 [PMID: 37098381] (Human)

Lafleur VN, Halim S, Choudhry H et al. Multi-level interaction between HIF and AHR transcriptional pathways in kidney carcinoma Life science alliance 2023-04-01 [PMID: 36725335] (IP, ChIP, Human, Mouse)

Yang Y, Chen C, Zuo Q Et al. NARF is a hypoxia-induced coactivator for OCT4-mediated breast cancer stem cell specification Sci Adv 2022-12-09 [PMID: 36490339] (Chemotaxis, Human)

Details:
Citation using the DyLight 405 version of this antibody.

Salman S, Meyers DJ, Wicks EE Et al. HIF inhibitor 32-134D eradicates murine hepatocellular carcinoma in combination with anti-PD1 therapy J Clin Invest 2022-05-02 [PMID: 35499076] (Chemotaxis, Human)

Details:
Citation using the Alexa Fluor 488 version of this antibody.

More publications at http://www.novusbio.com/NB100-110

Procedures

Protocol specific for HIF-1 beta Antibody (NB100-110)

Western Blot Procedure

1. Resolve aliquots (15 mg) of induced * nuclear protein extracts on a SDS/6% polyacrylamide gel.

2. Transfer to nitrocellulose membranes in 20 mM Tris-HCl (pH 8.0)/150 mM glycine/20% (vol/vol) methanol.

3. Block membranes for 1.5 hours with 1X western wash buffer containing 5% non-fat dry milk (NFDM).

4. Incubate membranes for 1.5 hours at room temperature (RT) in NB100-110 diluted 1:2,000 ** in 1X western wash/5% NFDM.

5. Wash with 1X western wash for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).

6. Incubate membranes with HRP conjugated anti-Rabbit IgG for 1 hour (RT) in 1X western wash/5% NFDM. Wash with 1X western wash for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).

7. Drain membrane and place on saran wrap.

8. Using Amersham ECL Kit, mix equal volumes of two reagents. Pour over membrane (protein side facing up). Let solution sit on membrane for 15-20 seconds.

9. Drain membrane and place on new saran wrap

10. Wrap up membrane and expose to film.

11. Develop accordingly.

Notes: If hypoxia treatment is not hypoxic enough (less than 2% oxygen to get an induction), signal will be absent. Also, if the harvest time is too slow or there are not enough protease inhibitors, etc., the induced protein will be rapidly lost as HIF-1beta has a very short half-life. Whole cell extracts or nuclear extracts of hypoxia induced cell lines (293, Hep3B, COS7, Hepa) are useful as a positive control. Nuclear Extract Preparation Reference: Wang and Semenza. "Purification and Characterization of Hypoxia-Inducible Factor 1". Journal of Biological Chemistry. 270(3): 1230-1237, 1995.

IHC-FFPE sections

I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:
A. Place slides in peroxidase quenching solution: 15-30 minutes.
To Prepare 200 ml of Quenching Solution:
Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.

C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:
-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
-Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
-All steps in which Xylene is used should be performed in a fume hood.
-For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.

-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.

-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.

-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.

-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 1/2 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

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Products Related to NB100-110

NBP3-11826	HIF-1 alpha Knockout CoCl2-treated/untreated HeLa Cell Lysate
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NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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