

# Product Datasheet

## AHR Antibody - BSA Free NB100-2289

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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**NB100-2289**

AHR Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	96 kDa

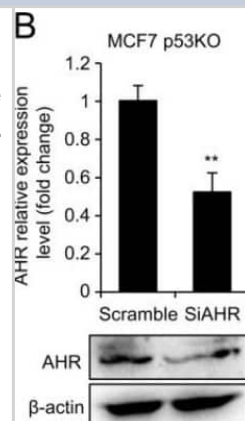
Product Description	
Host	Rabbit
Gene ID	196
Gene Symbol	AHR
Species	Human, Mouse, Rat, Guinea Pig
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 23887904). Porcine reactivity reported from a verified customer review.
Immunogen	Bacterially expressed human Aryl hydrocarbon Receptor (C-terminus). [UniProt# P35869]

Product Application Details	
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockdown Validated
Recommended Dilutions	Western Blot 1:500-1:2000, Simple Western 1:200, Flow Cytometry 2.5 ug/ml, ELISA 1:100 - 1:2000, Immunohistochemistry 1:100, Immunocytochemistry/Immunofluorescence 1:500-1:1000, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:100, Knockdown Validated
Application Notes	<p>In Western blot a band is seen ~90 to 105 kDa representing AHR (molecular weight varies by species and by strain). Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.</p> <p>The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.</p>

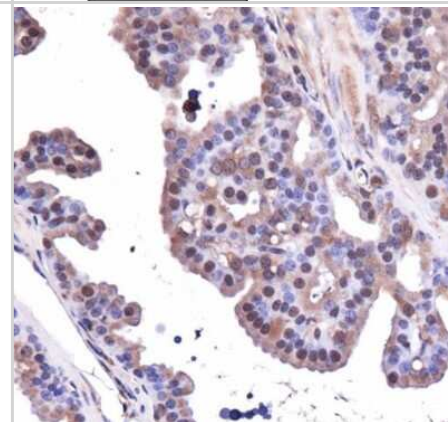


## Images

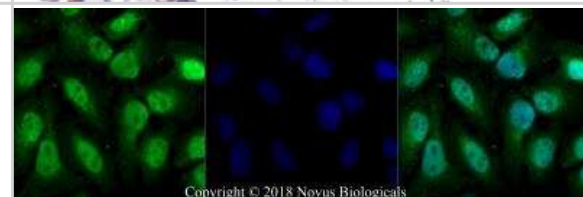
Knockdown Validated: AHR Antibody [NB100-2289] - Activation of AHR/Nrf2 pathway by PFT-alpha. Knock-down efficiency of AHR siRNA as detected by qPCR (upper part) and western blot (lower part). Relative expression level is shown as fold change normalized to scramble siRNA. All values represent the mean +/- SD of two independent experiments performed in three replicates. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-020-58051-1>), licensed under a CC-BY license.



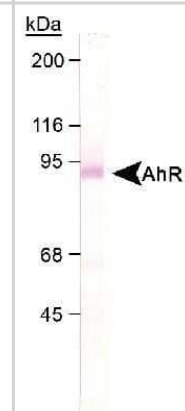
Immunohistochemistry: AHR Antibody [NB100-2289] - Staining of Aryl hydrocarbon Receptor in mouse prostate.



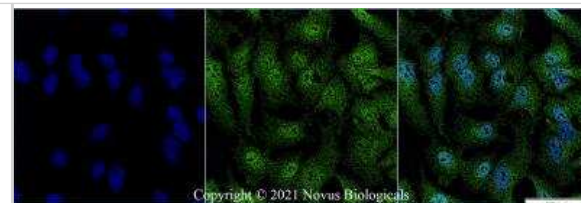
Immunocytochemistry/Immunofluorescence: AHR Antibody [NB100-2289] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton X-100. The cells were incubated with anti-AHR at 5 ug/mL overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



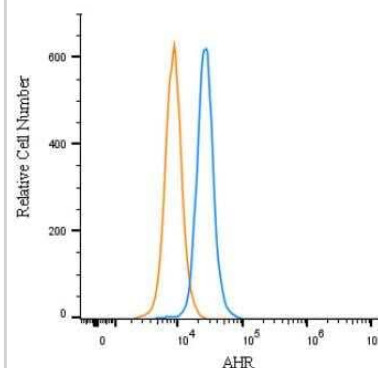
Western Blot: AHR Antibody [NB100-2289] - Detection of AhR in mouse liver cytosol using NB 100-2289.



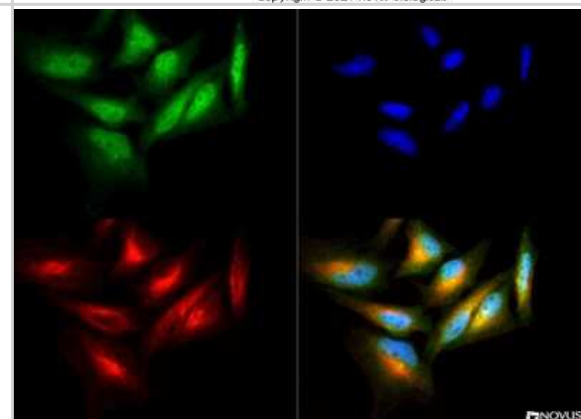
**Immunocytochemistry/Immunofluorescence: AHR Antibody [NB100-2289]** - HepG2 cells were fixed for 10 minutes using 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-AHR at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



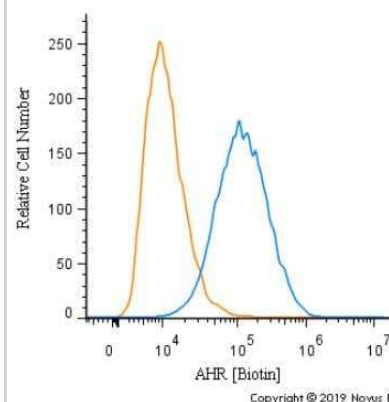
**Flow Cytometry: AHR Antibody [NB100-2289]** - An intracellular stain was performed on U937 cells with NB100-2289 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, DyLight 550 (SA5-10033, Thermo Fisher).



**Immunocytochemistry/Immunofluorescence: AHR Antibody [NB100-2289]** - Aryl hydrocarbon Receptor antibody was tested in HeLa cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



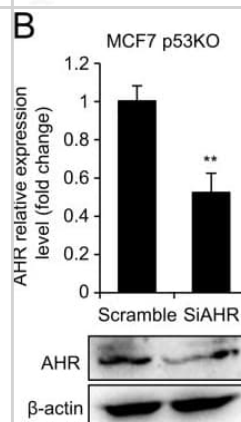
**Flow Cytometry: AHR Antibody [NB100-2289]** - An intracellular stain was performed on U-87 cells with AHR Antibody NB100-2289B (blue) and a matched isotype control (orange). Both antibodies were conjugated to Biotin. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Streptavidin - R-Phycoerythrin Protein (2012-1000, Novus Biologicals).



Simple Western: AHR Antibody [NB100-2289] - Image shows a specific band for Aryl hydrocarbon receptor in 0.5 mg/mL of HepG2 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



Activation of AHR/Nrf2 pathway by PFT-alpha. (B) Knock-down efficiency of AHR siRNA as detected by qPCR (upper part) and western blot (lower part). Relative expression level is shown as fold change normalized to scramble siRNA. All values represent the mean  $\pm$  SD of two independent experiments performed in three replicates. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31974452>), licensed under a CC-BY licence.



## Publications

Xie Z, Li M, Qian M et al. Co-Cultures of Lactobacillus acidophilus and Bacillus subtilis Enhance Mucosal Barrier by Modulating Gut Microbiota-Derived Short-Chain Fatty Acids Nutrients 2022-10-25 [PMID: 36364738] (WB)

Luo JM, Zhang TT, He YY et al. Human Chorionic Gonadotropin-Stimulated Interleukin-4-Induced-1 (IL4I1) Promotes Human Decidualization via Aryl Hydrocarbon Receptor International journal of molecular sciences 2023-02-05 [PMID: 36834576] (WB, Human)

Wang Y, Sun Q, Ye Y et al. FGF-2 signaling in nasopharyngeal carcinoma modulates pericyte-macrophage crosstalk and metastasis JCI insight [PMID: 35439170] (Chemotaxis, Human)

Baban B, Braun M, Khodadadi H, et al. AMPK induces regulatory innate lymphoid cells after traumatic brain injury JCI insight 2021-01-11 [PMID: 33427206] (FLOW, Human)

Zhu J, Singh M, Selivanova G, Peugeot S Pifithrin-alpha alters p53 post-translational modifications pattern and differentially inhibits p53 target genes Sci Rep 2020-01-23 [PMID: 31974452] (WB, Human)

Hsu CN, Lin YJ, Tain YL Maternal Exposure to Bisphenol A Combined with High-Fat Diet-Induced Programmed Hypertension in Adult Male Rat Offspring: Effects of Resveratrol Int J Mol Sci 2019-09-06 [PMID: 31489946] (WB, Rat)

Meng Y, Lin R, Wu F et al. Decreased Capacity for Sperm Production Induced by Perinatal Bisphenol A Exposure Is Associated with an Increased Inflammatory Response in the Offspring of C57BL/6 Male Mice. Int J Environ Res Public Health. 2018-10-01 [PMID: 30275377] (WB, Mouse)

Hsu CN, Lin YJ, Lu PC, Tain YL. Maternal Resveratrol Therapy Protects Male Rat Offspring against Programmed Hypertension Induced by TCDD and Dexamethasone Exposures: Is It Relevant to Aryl Hydrocarbon Receptor. Int J Mol Sci 2018-08-20 [PMID: 30127255] (WB, Rat)

Bradley JM, Cryar KA, El Hajj MC et al. Exposure to Diesel Exhaust Particulates Induces Cardiac Dysfunction and Remodeling. J Appl Physiol 2013-07-25 [PMID: 23887904] (WB, Rat)

## Procedures

### Western blot Protocol for Aryl hydrocarbon Receptor antibody (NB100-2289)

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

### ICC/IF Protocol for Aryl hydrocarbon Receptor antibody (NB100-2289)

#### Immunocytochemistry Protocol

Culture cells to appropriate density on suitable glass coverslips in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 5-10 minutes.
2. Remove the formalin and add 0.5% Triton-X 100 in TBS to permeabilize the cells. Incubate for 5-10 minutes.
3. Remove the permeabilization buffer and add wash buffer (i.e. PBS or PBS with 0.1% Tween-20). Be sure to not let the specimen dry out. Gently wash three times for 10 minutes.
4. Alternatively, cells can be fixed with -20C methanol for 10 min at room temperature. Remove the methanol and rehydrate in PBS for 10 min before proceeding.
5. To block nonspecific antibody binding incubate in 10% normal goat serum for 1 hour at room temperature.
6. Add primary antibody at appropriate dilution and incubate at room temperature for 1 hour or at 4 degrees C overnight.
7. Remove primary antibody and replace with wash buffer. Gently wash three times for 10 minutes.
8. Add secondary antibody at the appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove antibody and replace with wash buffer. Gently wash three times for 10 minutes.
10. Nuclei can be staining with 4',6' diamino phenylindole (DAPI) at 0.1 ug/ml, or coverslips can be directly mounted in media containing DAPI.
11. Cells can now be viewed with a fluorescence microscope.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow proper laboratory procedures for the disposal of formalin.

## IHC Protocol for Aryl hydrocarbon Receptor antibody (NB100-2289)

### Immunohistochemistry-Paraffin Embedded Sections

#### Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

#### Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 degrees C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.





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### **Products Related to NB100-2289**

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NB820-59662	Mouse Liver Whole Tissue Lysate (Adult Whole Normal)
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **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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