Product Datasheet

Carbonic Anhydrase IX/CA9 Antibody
NB100-417

Unit Size: 0.1 ml
Store at -20C. Avoid freeze-thaw cycles.

www.novusbio.com  technical@novusbio.com

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# NB100-417
Carbonic Anhydrase IX/CA9 Antibody

## Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 ml</th>
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</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1.0 mg/ml</td>
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<tr>
<td><strong>Storage</strong></td>
<td>Store at -20C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.025% Sodium Azide</td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Immunogen affinity purified</td>
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<tr>
<td><strong>Buffer</strong></td>
<td>PBS</td>
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<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>55 kDa</td>
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## Product Description

**Host**
Rabbit

**Gene ID**
768

**Gene Symbol**
CA9

**Species**
Human, Mouse, Rat, Plant

**Reactivity Notes**
Reactivity to various species reported in scientific literature (Species - PMID: Human - 31311071, Mouse - 31097477, Rat - 25767292, and Plant - 19278636).

**Marker**
Hypoxia Marker

**Immunogen**
This Carbonic Anhydrase IX/CA9 Antibody was made from a synthetic peptide from the C-terminal sequence of human Carbonic Anhydrase IX (within residues 400-459) [UniProt# Q16790].

## Product Application Details

**Applications**
Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunoblotting, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Dual RNAscope ISH-IHC

**Recommended Dilutions**

**Application Notes**
This Carbonic Anhydrase IX antibody is useful for Western blot, Immunofluorescence/Immunocytochemistry, and Immunohistochemistry. Gel Super Shift Assays was reported in scientific literature. In Western blot a band is observed approx. 53 kDa. Use in ELISA reported in scientific literature (PMID 19963243). Use in chromatin immunoprecipitation reported in multiple pieces of scientific literature. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue. 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.

Immunoblotting: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Hypoxias impact on proteins of the mitochondrial ISC assembly machinery. (A) Immunoblotting analyzed the total protein extracts from HeLa cells grown in normoxia (Nx, 21% O2) or hypoxia (Hx, 1% O2) conditions using VDACs poly antibody and anti-CAIX, -ISCU, -FXN, -NFS1, -HSC20 antibodies. Beta-Actin was used as loading control. Citation: Ferecatu I, Canal F, Fabbri L, Mazure NM, Bouton C, Golinelli-Cohen M-P (2018) Dysfunction in the mitochondrial Fe-S assembly machinery leads to formation of the chemoresistant truncated VDAC1 isoform without HIF-1 alpha activation. PLoS ONE 13(3): e0194782.

Immunohistochemistry: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Immunohistochemical representative microphotographs representing the HIF-1alpha, GLUT-1, and CAIX expression in endometrial cancer according to FIGO classification (IA, IB, II, IIIA, IIIC, and IV). Primary objective magnification 20x. Image collected and cropped by CiteAb from the following publication (http://www.hindawi.com/journals/bmri/2014/616850/) licensed under a CC-BY licence.

Western Blot: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Analysis in 1) HeLa, 2) MDA-MB-231, and 3) A549 whole cell lysates. Specific bands were detected for Carbonic Anhydrase IX/CA9 at a molecular weight of 50 kDa.


Immunohistochemistry-Paraffin: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - IHC analysis of a FFPE tissue section of human breast cancer using CAIX antibody at 1:1000 dilution. The primary antibody bound to CAIX antigens in the tissue section was detected using a HRP labeled secondary antibody and DAB reagent. Nuclei of the cells were counterstained with hematoxylin. This CAIX antibody generated an expected cytoplasmic staining of CAIX protein with an intense signal around the cellular membranes in tumor cores. The latter are more likely to be hypoxic in growing tumors which signifies that the observed CAIX staining is specific.

Simple Western: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Simple Western lane view shows a specific band for CAIX in 0.1 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Western Blot: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Analysis on various human cell lysates. Specific bands were detected for Carbonic Anhydrase IX/CA9 in HEK-293 and A498 cell lines at a molecular weight of approximately 50 kDa. WB image submitted by a verified customer review.

Immunocytochemistry/Immunofluorescence: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Immunocytochemical analysis of CNHCs with antibodies against the RCC marker CAIX. Clusters of CNHCs cytomorphologically classified as uncertain malignant (-UMF) with cytoplasmic positive staining with antibodies against the RCC marker CAIX. Image collected and cropped by CiteAb from the following publication (http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-11-214) licensed under a CC-BY licence.

Flow Cytometry: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - An intracellular stain was performed on U-87 MG Cells with NB100-417 and a matched isotype control. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 μg/mL for 30 minutes at room temperature, followed by Rabbit IgG APC-conjugated Secondary Antibody, (R&D Systems, F0111).
Western Blot: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Analysis in transfected HEK cell lysate using NB100-417. Rabbit IgG was used as a negative control.

Western Blot: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Analysis on rat renal cortex. A specific band was detected at a molecular weight of approximately 50 kDa.

Immunocytochemistry/Immunofluorescence: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Analysis of HRP conjugate of NB100-417. HEK 293 cells using NB100-417. Panel A shows CAIX-transfected cells (epitope-unmasked with SDS) and panel B shows mock transfected cells.

Immunocytochemistry/Immunofluorescence: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% TritonX-100. The cells were incubated with anti-CAIX at 2 µg/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.
Immunohistochemistry: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Analysis of renal carcinoma tissue. Staining using the Biotin conjugate, NB100-417B.

Immunoblotting: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Immunoblotting was used to determine CAIX/Carbonic Anhydrase IX/CA9 and other antibodies protein levels in total extracts. Cells were transfected using the specified siRNA for 6 days or treated with desferrioxamine (DFO) for 16 hours. Citation: Ferecatu I, Canal F, Fabbri L, Mazure NM, Bouton C, Golinelli-Cohen M-P (2018) Dysfunction in the mitochondrial Fe-S assembly machinery leads to formation of the chemoresistant truncated VDAC1 isoform without HIF-1 alpha activation. PLoS ONE 13(3): e0194782.

Immunoblotting: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Iron depletion and nitric oxide stress and Iron depletion induce the truncated VDAC1 form accumulation. (D) Total protein extracts were analyzed by immunoblotting using VDACs poly antibody and anti-HIF-1 alpha and -CAIX antibodies. (E) Immunoblotting was used to analyze total protein extracts using antibodies against the three VDAC isoforms. (F) HeLa cells were grown in hypoxia (Hx, 1% O2) conditions and transfected with iscu- or NC-siRNA for 3 days, or grown in normoxia (Nx, 21% O2), some treated with DFO for 16 h. Western Blots analyzed total proteins using VDACs poly antibody and anti-HIF-1 alpha, -CAIX, -ISCU antibodies. Citation: Ferecatu I, Canal F, Fabbri L, Mazure NM, Bouton C, Golinelli-Cohen M-P (2018) Dysfunction in the mitochondrial Fe-S assembly machinery leads to formation of the chemoresistant truncated VDAC1 isoform without HIF-1 alpha activation. PLoS ONE 13(3): e0194782.

Dual RNAscope ISH-IHC: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Formalin-fixed paraffin-embedded tissue sections of human stomach were probed for Carbonic Anhydrase IX/CA9 mRNA (ACD RNAscope Probe, catalog # 559348; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using Rabbit Polyclonal Carbonic Anhydrase IX/CA9 Antibody (Novus catalog # NB100-417) at 1:1000 dilution with overnight incubation at 4 degrees Celsius followed by incubation with anti-rabbit IgG VisUCyte HRP Polymer Antibody (Catalog # VC003) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to glandular cells.
<table>
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<tr>
<th>Publication</th>
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<tr>
<td>Perera RKS Investigating the role of the tumor microenvironment on tumor cell invasion and lymphatic dissemination Thesis (IHC, Mouse)</td>
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<tr>
<td>Kitson SJ, Maskell Z, Sivalingam VN et al. Optimization of Window Study Endpoints in Endometrial Cancer Front Oncol May 29 2019 12:00AM [PMID: 31214492]</td>
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<tr>
<td>Ron A, Dean-Ben XL, Gottschalk S, Razansky, D Volumetric optoacoustic imaging unveils high-resolution patterns of acute and cyclic hypoxia in a murine model of breast cancer Cancer Res. May 16 2019 12:00AM [PMID: 31097477] (IHC, Mouse)</td>
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<td>Reijnen, C;van Weelden, WJ;Arts, MSJP;Peters, JP;Rijken, PF;van de Vijver, K;Santacana, M;Bronsert, P;Bulten, J;Hirschfeld, M;Colas, E;Gil-Moreno, A;Reques, A;Mancebo, G;Krakstad, C;Trovik, J;Haldorsen, IS;Huvila, J;Koskas, M;Weinberger, V;Minar, L;Jandak Poor outcome in hypoxic endometrial carcinoma is related to vascular density Br. J. Cancer Apr 23 2019 12:00AM [PMID: 31011231] (IHC-P, Human)</td>
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<tr>
<td>Azam, SH;Porrello, A;Harrison, EB;Leslie, PL;Liu, X;Waugh, TA;Belanger, A;Mangala, LS;Lopez-Berestein, G;Wilson, HL;McCann, JV;Kim, WY;Sood, AK;Liu, J;Dudley, AC;Pecot, CV;Quaking orchestrates a post-transcriptional regulatory network of endothelial cell cycle progression critical to angiogenesis and metastasis Oncogene Mar 27 2019 12:00AM [PMID: 30918328] (IHC-Fr, Mouse)</td>
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Procedures

**Immunohistochemistry protocol for Carbonic Anhydrase IX Antibody (NB100-417)**

**Immunohistochemistry Procedure**

1. Using paraffin-embedded tissue sections, dewax and rehydrate the tissue, as per standard protocol.
2. Block the slides with peroxidase for 5 minutes.
3. Incubate slides with primary antibody [NB 100-417], 1:1,000 diluted in 10% normal serum, for 30 minutes at room temperature (RT).
4. Wash slides for 5 minutes.
5. Incubate slides with secondary antibody [envision, DAKO] for 30 minutes at RT.
6. Wash slides for 5 minutes.
7. Incubate slides with DAB chromagen for 6 minutes.
8. Counterstain slides with Hematoxylin. NOTE: Antigen retrieval was not used.

**Western Blot protocol (NB100-417)**

**Western Blot Protocol**

1. Perform SDS-PAGE on samples to be analyzed, loading 25 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%.
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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