Product Datasheet
Carbonic Anhydrase IX/CA9 Antibody - BSA Free
NB100-417

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

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## NB100-417
Carbonic Anhydrase IX/CA9 Antibody - BSA Free

### Product Information

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
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<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
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<tr>
<td>Storage</td>
<td>Store at -20°C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.02% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
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<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
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<tr>
<td>Buffer</td>
<td>PBS</td>
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<tr>
<td>Target Molecular Weight</td>
<td>55 kDa</td>
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### Product Description

**Host** | Rabbit  
**Gene ID** | 768  
**Gene Symbol** | CA9  
**Species** | Human, Mouse, Rat, Plant  
**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
- ELISA
- Flow Cytometry
- Gel Super Shift Assays
- Immunoblotting
- Immunocytochemistry/Immunofluorescence
- Immunohistochemistry
- Immunohistochemistry-Frozen
- Immunohistochemistry-Paraffin
- Immunoprecipitation
- Microarray
- Proximity Ligation Assay
- Chromatin Immunoprecipitation (ChIP)
- Dual RNAscope ISH-IHC

#### Recommended Dilutions

- Western Blot 1 - 3 ug/ml, Simple Western 1:50, Flow Cytometry 1:1000, ELISA reported in scientific literature (PMID 19963243), Immunohistochemistry 1:200 - 1:500, Immunocytochemistry/Immunofluorescence 2 - 5 ug/ml, Immunoprecipitation 1:10 - 1:500. Use reported in multiple pieces of scientific literature, Immunohistochemistry-Paraffin 1:200 - 1:500, Immunohistochemistry-Frozen 1:200 - 1:500, Immunoblotting, Gel Super Shift Assays, Proximity Ligation Assay, Microarray reported in scientific literature (PMID 31955345), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Dual RNAscope ISH-IHC 1:1000

#### Application Notes

In Western blot a band is observed approx. 53 kDa. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.
CA9 expressed in hypoxic regions as assessed by pimonidazole staining (A), but also observed in non-pimonidazole areas (B). Most of CA9 expressed in pimonidazole positive regions (C). Proliferation (BrdUrd) & apoptosis (caspase-3) in relation to CA9 expression shown in figure D & E. Red, CA9; Green, pimonidazole (A-B), BrdUrd (D) or caspase-3 (E); Yellow, overlap of CA9 (red) & pimonidazole (green); Light blue, vessels. Magnification 100x. Scale bars represent 100 um. Closed circles represent CA9 expression in pimonidazole positive regions; open circles represent CA9 expression in pimonidazole -ve regions. Image collected & cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0108068), licensed under a CC-BY license.

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 (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.

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.

Immunohistochemical representative microphotographs representing the HIF-1alpha, GLUT-1, and CAIX expression in endometrial cancer according to FIGO classification (IA, IB, II, IIA, IIC, 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 license.
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.

Analysis using the DyLight 488 conjugate of NB100-417. Staining of Carbonic Anhydrase IX (red) in human glioma U87 cells. DAPI counterstains nuclei (blue). ICC/IF image submitted by a verified customer review.

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.

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 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG APC-conjugated Secondary Antibody, (R&D Systems, F0111).
Western Blot analysis of Carbonic Anhydrase IX/CA9 Antibody on human breast cancer MCF7 cells. Image from verified customer review.

A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-Carbonic Anhydrase IX/CA9 Antibody NB100-417 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.

An intracellular stain was performed on A431 cells with Carbonic Anhydrase IX/CA9 Antibody NB100-417 (blue) and a matched isotype control (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).

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.
Analysis on rat renal cortex. A specific band was detected at a molecular weight of approximately 50 kDa.

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 license.

Analysis of human colon carcinoma, xenografted in mice. IHC-Fr image submitted by a verified customer review.

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.

Immunochemistry of human RCC tumor cryosections using NB100-417 (Panel A). Panel B shows staining with normal rabbit serum.

Colorectal cancer xenograft growth was suppressed by CB-PIC (20 and 50 mg/kg body weight) in female athymic nude mice. Starting three days after SW620 cell inoculation, CB-PIC (20 and 50 mg/kg body weight) was injected in abdomen with 4% Tween 20 as vehicle once daily. Representative examples of immunohistochemical staining for CA IX in tumor section. Image collected and cropped by CiteAb from the following publication (http://www.hindawi.com/journals/ecam/2013/974313/), licensed under a CC-BY license.

Iron depletion and nitric oxide stress 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.
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<tr>
<td>Aoki T, Kinoshita J, Munesue S et al. Hypoxia-Induced CD36 Expression in Gastric Cancer Cells Promotes Peritoneal Metastasis via Fatty Acid Uptake Annals of Surgical Oncology 2023-05-01 [PMID: 36042102] (IHC)</td>
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<td>Maurer GD, Tichy J, Harter PN et al. Matching Quantitative MRI Parameters with Histological Features of Treatment-Naïve IDH Wild-Type Glioma Cancers (Basel) 2021-08-12 [PMID: 34439213] (IHC-P, IHC)</td>
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<tr>
<td>Chow E, Lau JSH, Wai T, Lam IPY. The anti-tumoral effects of the oxygen carrier YQ23 in a triple-negative breast cancer syngeneic model Translational Cancer Research 2021-02-01 [PMID: 35116399]</td>
</tr>
<tr>
<td>Griesinger AM, Riemondy K, Eswaran N et al. Multi-omic approach identifies hypoxic tumor-associated myeloid cells that drive immunobiology of high-risk pediatric ependymoma iScience 2023-09-15 [PMID: 37694144]</td>
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<td>Glassman D, Kim MS, Spradlin M et al. Exploiting metabolic vulnerabilities after anti-VEGF antibody therapy in ovarian cancer iScience 2023-02-17 [PMID: 36824283]</td>
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<tr>
<td>Huang BR, Liu YS, Lai SW et al. CAIX Regulates GBM Motility and TAM Adhesion and Polarization through EGFR/STAT3 under Hypoxic Conditions International Journal of Molecular Sciences 2020-08-14 [PMID: 32823915]</td>
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### Immunohistochemistry protocol for Carbonic Anhydrase IX Antibody (NB100-417)

**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 (keep slides in the sodium citrate buffer all the time).

**Staining:**

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4°C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.

### Western Blot protocol for Carbonic Anhydrase IX/CA9 Antibody (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%.
Flow (Intracellular) Protocol for Carbonic Anhydrase IX/CA9 Antibody (NB100-417)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.
1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 10^5 and 1 x 10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
   a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Resuspend cells to a concentration of 1 x 10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.
Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:
1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabization buffer to every 1 x 10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
   a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
   b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.
Immunocytochemistry/Immunofluorescence Protocol for Carbonic Anhydrase IX/CA9 Antibody (NB100-417)

Immunocytochemistry Protocol

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.
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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