Product Datasheet

Carbonic Anhydrase IX/CA9 Antibody
NB100-417

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

Reviews: 4  Publications: 150

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Updated 8/22/2018 v.20.1

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## Product Information

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at -20C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.025% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<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

<table>
<thead>
<tr>
<th>Host</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene ID</td>
<td>768</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>CA9</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat, Canine, Plant</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>Human, dog, rat and mouse (PMID 22842475). Plant reactivity reported in scientific literature (PMID: 19278636)</td>
</tr>
<tr>
<td>Marker</td>
<td>Hypoxia Marker</td>
</tr>
<tr>
<td>Immunogen</td>
<td>A synthetic peptide made to a C-terminal sequence of human Carbonic Anhydrase IX (within residues 400-459) [UniProt Q16790]</td>
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## Product Application Details

<table>
<thead>
<tr>
<th>Applications</th>
<th>Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Gel Super Shift Assays, Immunoblotting, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application Notes</td>
<td>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.</td>
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</tbody>
</table>
Immunohistochemistry-Paraffin: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - IHC analysis of a formain fixed paraffin embedded (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.

Western Blot: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Analysis in 1) HeLa, 2) MDA-MB-231 and 3) A549 whole cell lysates.


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.


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 using the HRP conjugate. Analysis in transfected HEK cell lysate. Rabbit IgG was used as a negative control.
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% Triton-X100. The cells were incubated with anti-CAIX at 2 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.

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 ug/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 NB 100-417. Rabbit IgG was used as a negative control.

Western Blot: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Analysis on rat renal cortex.
Western Blot: Carbonic Anhydrase IX/CA9 Antibody [NB100-417] - Analysis on various human cell lysates. Image from verified customer review.


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.
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.


<table>
<thead>
<tr>
<th>Publications</th>
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<tbody>
<tr>
<td>Rosenberg T, Aaberg-Jessen C, Petterson SA, Kristensen BW. Heterogenic expression of stem cell markers in patient-derived glioblastoma spheroid cultures exposed to long-term hypoxia CNS Oncol Apr 1 2018 12:00AM [PMID: 29708435] (IHC, Human)</td>
</tr>
<tr>
<td>Ferecatu I, Canal F, Fabbri L et al. Dysfunction in the mitochondrial Fe-S assembly machinery leads to formation of the chemoresistant truncated VDAC1 isoform without HIF-1a activation. PLoS ONE Mar 29 2018 12:00AM [PMID: 29596470] (WB, Human)</td>
</tr>
<tr>
<td>Details: CAIX antibody from Novus was used to look at the effect of desferrioxamine treatment on human cell lines with regards to CAIX protein levels in western blot</td>
</tr>
<tr>
<td>Wang D, Berglund AE, Kenchappa RS et al. BIRC3 is a biomarker of mesenchymal habitat of glioblastoma, and a mediator of survival adaptation in hypoxia-driven glioblastoma habitats Sci Rep 2017 Aug 24 [PMID: 28839258] (WB, Mouse)</td>
</tr>
</tbody>
</table>

## 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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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Products Related to NB100-417

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>NB800-PC1</td>
<td>HeLa Whole Cell Lysate</td>
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<tr>
<td>NB100-417PEP</td>
<td>Carbonic Anhydrase IX/CA9 Blocking Peptide</td>
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<tr>
<td>HAF008</td>
<td>Goat anti-Rabbit IgG Secondary Antibody [HRP (Horseradish Peroxidase)]</td>
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<tr>
<td>NB7156</td>
<td>Goat anti-Rabbit IgG (H+L) Secondary Antibody</td>
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<tr>
<td>NBP2-24891</td>
<td>Rabbit IgG Isotype Control</td>
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