Product Datasheet

N-Cadherin Antibody (13A9)
NBP1-48309

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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### NBP1-48309
N-Cadherin Antibody (13A9)

<table>
<thead>
<tr>
<th><strong>Product Information</strong></th>
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</thead>
<tbody>
<tr>
<td><strong>Unit Size</strong></td>
<td>0.1 ml</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Monoclonal</td>
</tr>
<tr>
<td><strong>Clone</strong></td>
<td>13A9</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.05% Sodium Azide</td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
<td>IgG1</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Protein G purified</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>Tris-Glycine and 0.15M NaCl</td>
</tr>
<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>140 kDa</td>
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<table>
<thead>
<tr>
<th><strong>Product Description</strong></th>
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<tbody>
<tr>
<td><strong>Host</strong></td>
<td>Mouse</td>
</tr>
<tr>
<td><strong>Gene ID</strong></td>
<td>1000</td>
</tr>
<tr>
<td><strong>Gene Symbol</strong></td>
<td>CDH2</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Human, Mouse, Rat</td>
</tr>
<tr>
<td><strong>Reactivity Notes</strong></td>
<td>Human and mouse.</td>
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<tr>
<td><strong>Marker</strong></td>
<td>Mesenchymal Cells Marker</td>
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<tr>
<td><strong>Immunogen</strong></td>
<td>Cytoplasmic domain of human N Cadherin [Swiss-Prot# P19022]</td>
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<table>
<thead>
<tr>
<th><strong>Product Application Details</strong></th>
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<tbody>
<tr>
<td><strong>Applications</strong></td>
<td>Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation</td>
</tr>
<tr>
<td><strong>Recommended Dilutions</strong></td>
<td>Western Blot 0.5 ug/ml, Simple Western 1:50, Flow Cytometry, Immunohistochemistry 1:50-1:200, Immunocytochemistry/Immunofluorescence 1:100, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:50-1:100</td>
</tr>
<tr>
<td><strong>Application Notes</strong></td>
<td>This N Cadherin (13A9) antibody is useful for Immunofluorescence/Immunocytochemistry, Immunoprecipitation, Immunohistochemistry on paraffin-embedded sections and Western Blot, where a band is observed at approx. 140 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. 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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Western Blot: N-Cadherin Antibody (13A9) [NBP1-48309] - Analysis of N Cadherin expression in HeLa whole cell lysate.

Immunocytochemistry/Immunofluorescence: N-Cadherin Antibody (13A9) [NBP1-48309] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti N-Cadherin (13A9) [NBP1-48309] at a 1:100 dilution overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

Immunohistochemistry: N-Cadherin Antibody (13A9) [NBP1-48309] - IHC analysis of N Cadherin in mouse liver using DAB with hematoxylin counterstain.

Flow Cytometry: N-Cadherin Antibody (13A9) [NBP1-48309] - An intracellular stain was performed on HeLa cells with N-Cadherin Antibody (13A9)NBP1-48309AF488 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 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.
Flow (Intracellular): N-Cadherin Antibody (13A9) [NBP1-48309] - An intracellular stain was performed on HeLa with N-Cadherin Antibody (13A9) NBP1-48309 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 1 ug/mL for 30 minutes at room temperature, followed by Mouse F(ab)2 IgG (H+L) PE-conjugated Antibody.

Flow Cytometry: N-Cadherin Antibody (13A9) [NBP1-48309] - An intracellular stain was performed on HeLa cells with N-Cadherin Antibody (13A9) NBP1-48309R 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 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Dylight 550.

Simple Western: N-Cadherin Antibody (13A9) [NBP1-48309] - Simple Western lane view shows a specific band for N Cadherin in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.


Details:
N-Cadherin antibody used for Immunocytochemistry on KCI-MENG1 cells or KCI-MENG1-LPSX cells, which are dissociated cells from second generation xenograft mouse tumor) at a dilution of 1:100.


**Procedures**

**Protocol specific for N Cadherin Antibody (13A9) [NBP1-48309]**

### Western Blot Protocol
1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 25 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the anti-N Cadherin primary antibody (NBP1-48309) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted mouse-IgG 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 [TBS + 0.1% Tween] 3 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 (Pierce ECL).

**Note:** Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05 -0.2%, provided it does not interfere with antibody-antigen binding.

### Immunohistochemistry-Paraffin Embedded Sections

**Antigen Unmasking:**

Bring slides to a boil in a 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 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.

### Immunocytochemistry Protocol

Culture cells to appropriate density 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 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.*
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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<td>NB800-PC1</td>
<td>HeLa Whole Cell Lysate</td>
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<tr>
<td>HAF007</td>
<td>Goat anti-Mouse IgG Secondary Antibody [HRP (Horseradish Peroxidase)]</td>
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<tr>
<td>NB720-B</td>
<td>Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin] (Pre-absorbed)</td>
</tr>
<tr>
<td>NBP1-97005</td>
<td>Mouse IgG1 Isotype Control</td>
</tr>
</tbody>
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Novus Biologicals USA
8100 Southpark Way, A-8
Littleton, CO 80120
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Novus Biologicals Canada
461 North Service Road West, Unit B37
Oakville, ON L6M 2V5
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada@novusbio.com

Novus Biologicals Europe
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com