Product Datasheet

Survivin Antibody
NB500-201

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

Reviews: 21  Publications: 337

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## Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.02% 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>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>PBS</td>
</tr>
<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>16 kDa</td>
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</tbody>
</table>

## Product Description

**Host**
Rabbit

**Gene ID**
332

**Gene Symbol**
BIRC5

**Species**
Human, Mouse, Rat, Canine, Feline, Guinea Pig, Hamster

**Reactivity Notes**
Hamster reactivity reported in scientific literature (PMID: 23405201). Guinea Pig reactivity reported in scientific literature (PMID: 21364656).

**Immunogen**
This Survivin Antibody was developed against full length recombinant human Survivin [UniProt# O15392]

## Product Application Details

**Applications**
Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Dual RNAscope ISH-IHC, Knockdown Validated

**Recommended Dilutions**

**Application Notes**
In WB, a band at approx. 16.5 kDa can be seen. For IHC, prior antigen retrieval (pressure cooking) is recommended for cytoplasmic and nuclear detection of Survivin. Immunohistochemistry-Frozen, Flow Cytometry, and ELISA were reported in scientific literature (PMIDs: 12671708, 17875988, 24102797).

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.
Dual RNAscope ISH-IHC: Survivin Antibody [NB500-201] - Formalin-fixed paraffin-embedded tissue sections of human esophagus squamous cell carcinoma were probed for Survivin mRNA (ACD RNAscope Probe, [465361]; Fast Red chromogen, ACD [322360]). Adjacent tissue section was processed for immunohistochemistry using rabbit polyclonal [NB500-201] at 1.5ug/mL with overnight incubation at 4 degrees Celsius followed by incubation with anti-rabbit IgG VisUCyte HRP Polymer Antibody [VC003] and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to tumor cells.

Western Blot: Survivin Antibody [NB500-201] - Analysis of 30ug of HeLa whole cell lysate [NB800-PC1] using rabbit polyclonal [NB500-201] at 1ug/ml. Detection was performed using ECL method with 1 minute exposure. Band detected at higher molecular weight than the predicted MW (16 kDa).

Immunocytochemistry/Immunofluorescence: Survivin Antibody [NB500-201] - Analysis using the HRP conjugate of [NB500-201]. Staining of Telophase with accumulation of survivin in the midbodies of two daughter cells. Survivin detection using [NB500-201].

Knockdown Validated: Survivin Antibody [NB500-201] - Anti-tumor effects and functional evidence of sCA-survivin-siRNA in HCT116 and HT29 solid tumor models. Immunostaining of survivin in the tumor tissues on day 19 using [NB500-201]. Scale bar, 50 um. Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0116022) licensed under a CC-BY licence.
Knockdown Validated: Survivin Antibody [NB500-201] - Aurora B phosphorylates EB2. (c,d) HeLa cells were transfected with siRNAs as indicated; after 24 h, the cells were treated with nocodazole for 4 h. Mitotic cells were harvested by mitotic shake-off. Image collected and cropped by CiteAb from the following publication (http://www.nature.com/doifinder/10.1038/ncomms11117), licensed under a CC-BY licence.


Flow Cytometry: Survivin Antibody [NB500-201] - An intracellular stain was performed on HeLa cells with [NB500-201] 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 (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550.

Western Blot: Survivin Antibody [NB500-201] - Western blot analysis using Survivin Antibody [NB500-201]. Effects of siRNA transfection on the expression of Bcl-xL and survivin. Bcl-xL and survivin protein content detected by Western Blotting 48 h after transfection. Bcl-xL and survivin levels are shown normalized to the reference protein beta-actin and relative to the ns-si control. Image collected and cropped by CiteAb from the following publication (http://www.mdpi.com/1422-0067/14/6/12297) licensed under a CC-BY licence.
Immunohistochemistry-Paraffin: Survivin Antibody [NB500-201] - Survivin shows lysates of human neuroblastoma cell line. Polyvinylidene fluoride (PVDF) membrane was probed with 1:200 dilution of 0.5 ug/mL of rabbit polyclonal Survivin Antibody [NB500-201], followed by 1:2000 dilution of goat anti-rabbit IgG.

Western Blot: Survivin Antibody [NB500-201] - Analysis of Survivin in human hepatocytes from cancer patient (left) and HeLa cell lysate (right) using [NB500-201]. Image from verified customer review. Note: bands detected at higher molecular weight than predicted (16 kDa)

Western Blot: Survivin Antibody [NB500-201] - Western blot analysis using [NB500-201]. Survivin protein is degraded by ubiquitin proteasome in serum-free and serum-containing media. Western blots and protein quantitation graphs showing that addition of MG-132 proteasome inhibitor (10 uM) extended survivin protein half-life in serum-free and serum-containing media. One representative western blot out of triplicate experiments was shown for each treatment and condition. *Indicates the time at which survivin protein is half of the amount at 0 hours (half-life), P < 0.02. Image collected and cropped by CiteAb from the following publication (http://www.hindawi.com/journals/grp/2012/897678/) licensed under a CC-BY licence.

Immunocytochemistry/Immunofluorescence: Survivin Antibody [NB500-201] - Analysis of HeLa cells using Survivin Antibody ([NB500-201], 1:10). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue).

Immunohistochemistry: Survivin Antibody [NB500-201] - HRP conjugated Survivin expression in BIRC5 transfected 293T cells using Survivin Antibody [NB500-201]. Image from verified customer review.

Immunohistochemistry: Survivin Antibody [NB500-201] - Immunohistochemical analysis using [NB500-201]. The top photo is a control stain and the bottom photo is anti-survivin staining of melanoma. Photo courtesy of Dr. Dario Altieri, Yale University.

Simple Western: Survivin Antibody [NB500-201] - Simple Western analysis using [NB500-201]. Lane view shows a specific band for Survivin in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system. Theoretical molecular weight: 16 kDa.
<table>
<thead>
<tr>
<th>Publication</th>
<th>Date</th>
<th>PMID</th>
<th>Species</th>
<th>Tissue</th>
<th>Description</th>
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<tr>
<td>Hayashi N, Asano K, Suzuki H et al Adenoviral infection of survivin antisense sensitizes prostate cancer cells to EPE in vivo Prostate 2005 Sep 15 [PMID: 15799033] (WB)</td>
<td>2005</td>
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More publications at [http://www.novusbio.com/NB500-201](http://www.novusbio.com/NB500-201)
Procedures

Western Blot protocol for Survivin Antibody (NB500-201)
Survivin Antibody: https://www.novusbio.com/products/survivin-antibody_nb500-201

Western Blot Procedure
1) Cells were pelleted, washed in 1XPBS, suspended in ice water (~ 5 x 10\(^6\) cells/ml), and placed on ice
2) Lysates were prepared with the addition of 2X lysis buffer [2% SDS/ 50mM Tris-HCl / 10% glycerol]
3) Lysates were heated to 95 degrees C for 3 minutes and then microfuged at room temperature for 10 minutes
4) 50 ug of lysate were electrophoresed (150 V) through a 4-15% PAGE
5) Proteins were transferred (60 V) onto an Immobilon-P membrane (Millipore Corp.) for 45 minutes
6) The blot was blocked overnight at 4 degrees C in blocking buffer [1XPBS, pH 7 / 5% nonfat milk / 0.1% Tween-20]
7) Washed the blot in 1XPBS / 0.1% Tween-20
8) Incubated the blot with 1 ug/ml of (NB500-201) anti-Survivin antibody, diluted in blocking buffer, for 2 hours at room temperature
9) Washed the blot in 1XPBS / 0.1% Tween-20
10) Reacted the blot with HRP-conjugated donkey anti-rabbit Ig, diluted in 1XPBS / 0.1% Tween-20, for 30 minutes at room temperature
11) Washed the blot in 1XPBS / 0.1% Tween-20
12) Visualized blot by ECL and autoradiography

NOTE: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.
Immunohistochemistry-Paraffin protocol for Survivin Antibody (NB500-201)
Survivin Antibody: https://www.novusbio.com/products/survivin-antibody_nb500-201

Materials

1) 1 Phosphate buffered saline (pH 7.6): NaCl 137mmol/L, KCl 2.7mmol/L, Na2HPO4 4.3mmol/L, KH2PO4 1.4 mmol/L
2) Citrate buffer, 0.01 M, pH6.0, Sodium Citrate 3g, Citric acid 0.4g
3) 3% Hydrogen peroxide
4) Primary antibody
5) Blocking serum (normal serum)
6) Biotinylated secondary antibody
7) DAB staining kit

Methods

1. Dewax and hydration of slides using xylene and EtOH:
   Dry slides for 20 min in a 60 C oven
   Add Xylene, 2 x 10 min
   100%, 95%, 80%, and 70% EtOH, 5 min each EtOH concentration
   Rinse in PBS, 5’

2 Antigen retrieval method (only for paraffin slides)
1a. High-pressure antigen retrieval procedure (recommended method)
   Place slides in a glass slide holder (ensure that the slide holder is completely filled with slides, slides without sections if necessary, to ensure even heating. The entire slide holder is immersed in 1000 ml of Citrate buffer (0.01M, pH6.0) within a pressure cooker
   Once steam is produced, and ONLY when steam is visible, from the pressure cooker (usually 15-20 min), the required high-pressure will have been reached, and slides will be incubated for 2 min.
   Turn off heat, and allow buffer and slides to cool to room temperature
   Slides are then rinsed in PBS for 5 minutes
2. Add 3% hydrogen peroxide solution, 10’at RT, then PBS, 3X5’
3. Normal blocking serum, 20’at RT
4. Incubate with Primary Ab, 4C overnight or 1.5 hours at 37C
5. Rinse with PBS, 3 X 5’ each rinse
6. Add Biotin-conjugated second antibody, 10’at RT
7. Rinse with PBS, 3 X 5’ each rinse
8. Add Streptavidin Peroxidase, 10’at RT
9. Rinse with PBS, 3 X 5’ each rinse
10. Staining with DAB solution, 2-5’under microscope
11. Stop the reaction by washing in tap water
12. Counterstain in Haematoxylin for 3-5 minutes
13. 75%, 80%, 95% and 100% ethanol, 5x2’, xylene 2 x 10’
Immunoprecipitation protocol for Survivin Antibody (NB500-201)

Survivin Antibody: https://www.novusbio.com/products/survivin-antibody_nb500-201

Immunoprecipitation Procedure
1) Lyse cells plated in a 60mm dish:
   a) 300 ul CHAPS buffer [50mM Tris-HCl, pH 7.5/50mM NaCl/1mM EDTA/1% NP-40/0.1% CHAPS/1mM NaVO4/1mM PMSF]
   b) Rock for 20 minutes at 4 degrees C
2) Harvest lysate and spin down the insoluble material at 14K rpm
3) Collect soluble fraction
4) Pre-clear lysate with 40 ul of 50:50 slurry of Protein A beads, rocking for 1 hour at 4 degrees C
5) Spin down beads at 2K rpm, at 4 degrees C
6) Collect pre-cleared lysate
7) Incubate lysate with 5-7ug of anti-Survivin (NB 500-201) overnight, rocking at 4 degrees C
8) Add 50 ul of Protein A 50:50 slurry for 2 hours, rocking at 4C
9) Wash beads with 200 ul of CHAPS buffer, three times
10) Denature immune complex by adding 2x Sample Buffer, containing 2-ME
11) Boil for 10 minutes and load onto an SDS-gel.
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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