

Product Datasheet

Survivin Antibody NB500-201SS

Unit Size: 0.025 ml

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

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Updated 6/15/2014 v.20.1

NB500-201SS

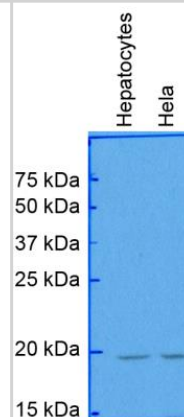
Survivin Antibody

Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	16.5 kDa
Product Description	
Host	Rabbit
Gene ID	332
Gene Symbol	BIRC5
Species	Human, Mouse, Rat, Canine, Feline, Guinea Pig, Hamster
Species Reactivity	Human, mouse, rat, feline, hamster, canine and guinea pig.
Immunogen	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
Recommended Dilutions	Chromatin Immunoprecipitation 1:10-1:500, Immunocytochemistry/Immunofluorescence 1:50-1:250, Immunohistochemistry 1:50-1:100, Immunohistochemistry-Paraffin 1:50-1:500, Immunoprecipitation 1:10-1:500, Western Blot 1 ug/ml, ELISA, Immunohistochemistry-Frozen, Flow Cytometry, Simple Western 1:25
Application Notes	This Survivin antibody is useful for Chromatin Immunoprecipitation, Immunocytochemistry/Immunofluorescence, Immunohistochemistry on paraffin-embedded sections, Immunoprecipitation and Western Blot. In WB, a band at ~16.5 kDa can be seen. For IHC, prior antigen retrieval (pressure cooking) is recommended for cytoplasmic and nuclear detection of Survivin. Immunohistochemistry-Frozen and Flow Cytometry were reported in scientific literature. In Simple Western only 10-15 uL of the recommended dilution is used per data point.

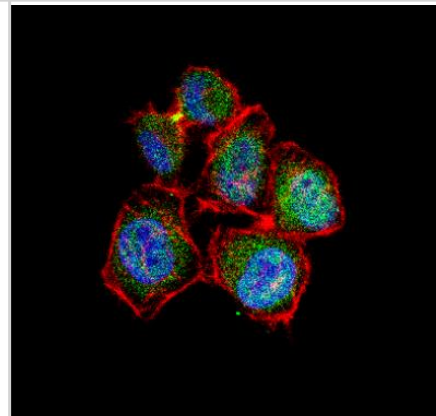


Images

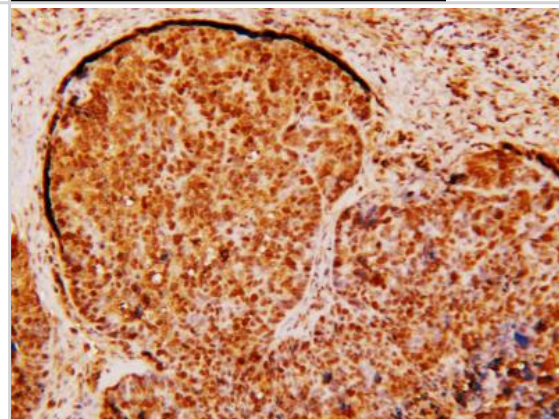
Western Blot: Survivin Antibody [NB500-201] - analysis of Survivin in human hepatocytes from cancer patient (left) and HeLa cell lysate (right) using Survivin antibody. Image from verified customer review.



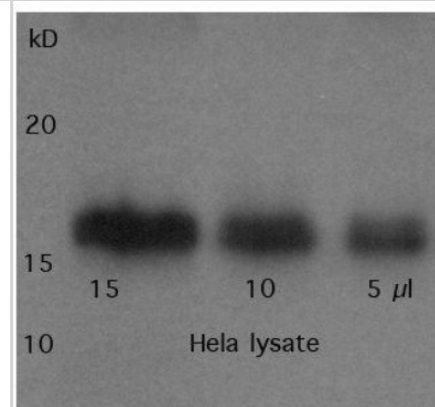
Immunocytochemistry/Immunofluorescence: Survivin Antibody [NB500-201] - IF Confocal analysis of HeLa cells using Survivin antibody (NB500-201, 1:10). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red).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



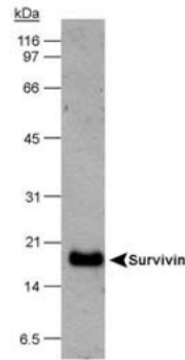
Immunohistochemistry-Paraffin: Survivin Antibody [NB500-201] - analysis of Survivin in ovarian cancer tissue using anti-Survivin antibody. Image from verified customer review.



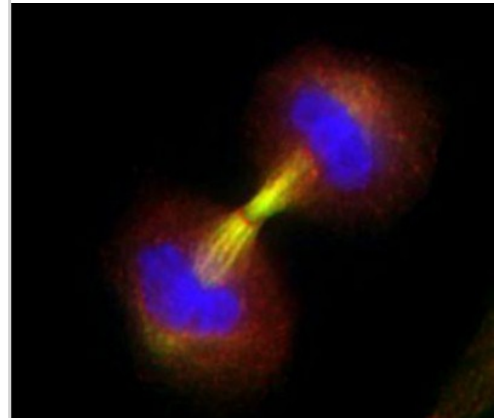
Western Blot: Survivin Antibody [NB500-201] - Analysis of Survivin in HeLa cell lysate. Image courtesy of anonymous customer review.



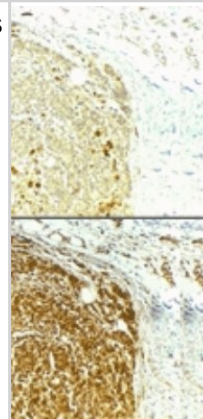
Western Blot: Survivin Antibody [NB500-201] - Detection of survivin in HeLa whole cell lysate (30 ug) using NB 500-201 at 1ug/ml (1 minute exposure).



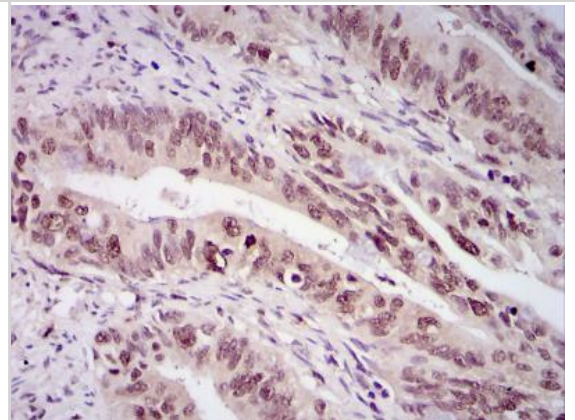
Immunocytochemistry/Immunofluorescence: Survivin Antibody [NB500-201] - Telophase with accumulation of survivin in the midbodies of two daughter cells.



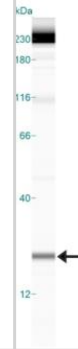
Immunohistochemistry: Survivin Antibody [NB500-201] - The top photo is a no primary antibody control stain and the bottom photo is anti-survivin staining of melanoma. Photo courtesy of Dr. Dario Altieri, Yale University.



Immunohistochemistry-Paraffin: Survivin Antibody [NB500-201] - IHC staining of Survivin in human rectal cancer using DAB with hematoxylin counterstain.



Simple Western: Survivin Antibody [NB500-201] - Simple Western 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.



Publications

Fu Dah-Renn, Kato Daiki, Watabe Ai et al. Prognostic utility of apoptosis index, Ki-67 and survivin expression in dogs with nasal carcinoma treated with orthovoltage radiation therapy. J Vet Med Sci. 2014 Aug 25 [PMID: 25452259] (IHC)

Kapinas Kristina, Kim Heesun, Mandeville Matthew et al. microRNA-mediated survivin control of pluripotency. J Cell Physiol. 2014 [PMID: 24891298] (Human)

Galloway Nicholas R, Diaz Osterman Carlos J, Reiber Karl et al. Yin Yang 1 regulates the transcriptional repression of Survivin. Biochem Biophys Res Commun. 2014 Feb 28 [PMID: 24508259] (WB, Human)

Yi XP, Han T, Li YX et al. Simultaneous silencing of XIAP and survivin causes partial mesenchymal-epithelial transition of human pancreatic cancer cells via the PTEN/PI3K/Akt pathway Mol Med Rep. 2015 Feb 20 [PMID: 25707849]

Cornago Protomartir M. Caracterizacion de los efectos de los inhibidores de las histona desacetilasas en lineas celulares de glioma Thesis. 2014 Sep 01 (WB, ICC/IF, Human)

Yamazaki H, Takagi S, Hosoya K. Survivin suppressor (YM155) enhances chemotherapeutic efficacy against canine histiocytic sarcoma in murine transplantation models Research in Veterinary Science. 2015 Feb 11 [PMID: 25744435] (IHC-P, Canine)

Details:

Survivin antibody used at 1:500 dilution in IHC-P application on sections of canine histiocytic sarcoma/HS xenografts from mice treated or not with survivin suppressor sepantronium bromide / YM155. Canine HS cell lines used in these experiments were CHS-4, CTT, and LHS and their derivatives CHS-4-CR, CTT-CR, and LHS-CR as CCNU-resistant HS cells (see full text for detailed protocols, data images shown in Fig 5a).

Lu H, Cao X, Chen Q et al. The Expression and Role of MEKK3 in Renal Clear Cell Carcinoma. ANat Rec (Hoboken). 2014 Nov 12 [PMID: 25388155]

Vogt N, Abramov D, Koch K et al. No evidence of cell cycle dysregulation in mantle cell lymphoma in vivo. Leuk. Lymphoma. 2014 Oct 15 [PMID: 25315075] (ICC/IF, Human)

Cornago M, Garcia-Alberich C, Blasco-Angulo N et al. Histone deacetylase inhibitors promote glioma cell death by G2 checkpoint abrogation leading to mitotic catastrophe. Cell Death Dis. 2014 Oct 03 [PMID: 25275596] (WB, ICC/IF, Human)

Details:

Survivin antibody used for ICC-IF on U251-MG cells in metaphase after HDAC inhibitor suberanilohydroxamic acid/SAHA treatment (Figure 7a). Antibody also used for WB on lysates of glioma cell lines U87, U251-MG, LN229 cells as well as human GBM primary cultures C55, C65, C48 and C60 treated or not with HDAC inhibitor SAHA and valproic acid/VPA (Figure 7b and c)

Grazia G, Vegetti C, Benigni F et al. Synergistic anti-tumor activity and inhibition of angiogenesis by cotargeting of oncogenic and death receptor pathways in human melanoma. Cell Death Dis. 2014 Oct 03 [PMID: 25275595] (WB, Human)

Details:

Survivin antibody used for WB on lysates of melanoma/mesothelioma cells lines Me13 and Me41 treated or not with AZD6244 (A), BEZ235 (B) TRAIL (T) and their combinations (AB, ABT). Blots shown in Figure 5a

Artesi M, Kroonen J, Bredel M et al. Connexin 30 expression inhibits growth of human malignant gliomas but protects them against radiation therapy. Neuro-oncology. 2014 Aug 25 [PMID: 25155356] (WB, Human)

Fu DR, Kato D, Watabe A et al. Prognostic Utility of Apoptosis Index, Ki-67 and Survivin Expression in Dogs with Nasal Carcinoma Treated with Orthovoltage Radiation Therapy. J Vet. Med. Sci. 2014 Aug 25 [PMID: 25151945] (IHC-P, Canine)

More publications at <http://www.novusbio.com/NB500-201>



Procedures

Western Blot Protocol Specific for Survivin Antibody (NB500-201)

Western Blot Procedure

- 1) Cells were pelleted, washed in 1XPBS, suspended in ice water (~ 5 x 10⁶ 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 Specific for 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 Specific for 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.





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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 guarantee, please visit www.novusbio.com/guarantee.

