# **Product Datasheet**

# Survivin Antibody (32.1) NB500-237

Unit Size: 0.1 ml

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

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# NB500-237

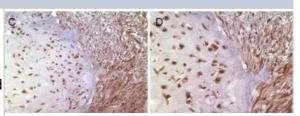
Survivin Antibody (32.1)	
Product Information	
Unit Size	0.1 ml
Concentration	1.9 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	32.1
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	16 kDa
Product Description	
Description	Novus Biologicals Mouse Survivin Antibody (32.1) - BSA Free (NB500-237) is a monoclonal antibody validated for use in IHC, WB, ELISA, Flow, ICC/IF and IP. Anti-Survivin Antibody: Cited in 11 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	332
Gene Symbol	BIRC5
Species	Human, Hamster
Reactivity Notes	Hamster reactivity reported in scientific literature (PMID: 23405201).
Specificity/Sensitivity	Survivin Antibody (32.1) [NB500-237] is specific for the nuclear form of survivin.
Immunogen	This Survivin Antibody (32.1) was developed against full length recombinant human Survivin [UniProt# O15392]
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000, Flow Cytometry, ELISA reported in scientific literature (PMID 11861764), Immunohistochemistry 20 ug/ml, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunoprecipitation reported in scientific literature (PMID 11861764), Immunohistochemistry-Paraffin 20 ug/ml
Application Notes	By WB, this antibody recognizes a band at ~16.5 kDa representing Survivin.  *The investigator should determine the optimal working dilution for a specific



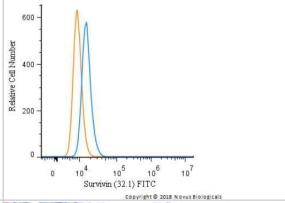
application.

## **Images**

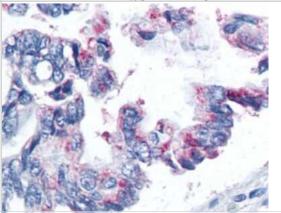
Immunohistochemistry-Paraffin: Survivin Antibody (32.1) [NB500-237] - Survivin expression in human chondrosarcoma. Immunohistochemistry and immunoblot for survivin (red staining) from human chondrosarcoma specimens. Specimen of a grade III chondrosarcoma stained with monoclonal antibody, shows a similar pattern of staining. Image collected and cropped by Citeab from the following publication (The antiapoptotic gene survivin is highly expressed in human chondrosarcoma and promotes drug resistance in chondrosarcoma cells in vitro. BMC Cancer (2011) licensed under a CC-BY license.



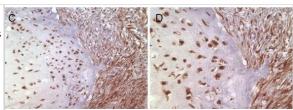
Flow Cytometry: Survivin Antibody (32.1) [NB500-237] - An intracellular stain was performed on HeLa cells with FITC-conjugated Survivin Antibody (32.1) [NB500-237F] (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.



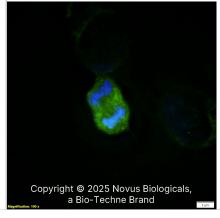
Immunohistochemistry-Paraffin: Survivin Antibody (32.1) [NB500-237] - Immunohistochemical staining of formalin-fixed paraffin-embedded human lung cancer tissue using Survivin Antibody (32.1) [NB500-237].



Western Blot: Survivin Antibody (32.1) - BSA Free [NB500-237] -Survivin expression in human chondrosarcoma. Immunohistochemistry & immunoblot for survivin (red staining) from human chondrosarcoma specimens. A: Low-power image of human high-grade chondrosarcoma displays strong cellular expression of survivin protein. B: High-power magnification reveals the predominantly cytoplasmic staining, although strong nuclear signals are detectable. C & D: Other specimen of a grade III chondrosarcoma stained with monoclonal antibody, shows a similar pattern of staining. E: Strong survivin signal in a tumor cell displaying a mitotic figure (arrow). F: To verify the expression of survivin in human chondrosarcoma, immunoblots were performed from 3 high grade chondrosarcoma lysates (Patient Nr. 5, 7, 10). As control for the correct molecular weight, in vitro-transcribed & -translated (IVTT) recombinant survivin protein, derived from the full-length human cDNA was loaded. Furthermore, lysates from adult human cartilage served as a negative control. Total protein loaded was 1 µg for recombinant human survivin, 60 µg for chondrosarcoma & cartilage lysates. For A, B & E the polyclonal rabbit anti-survivin antibody AF886 was used. For C & D the monoclonal mouse anti-survivin antibody clone 32.1 was used. Original magnifications: 200× (A & C) & 400× (B & D) & 600× (E). Image collected & cropped by CiteAb from the following publication (http://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-11-120), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Survivin (32.1) was detected in immersion fixed U-2 OS human osteosarcoma cell line using Mouse anti-Survivin (32.1) Protein G Purified Monoclonal Antibody conjugated to FITC (Catalog # NB500-237F) (green) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



#### **Publications**

Wu X, Li Y, Liu X et al. B7-H1(PD-L1) confers chemoresistance through ERK and p38 MAPK pathway in tumor cells bioRxiv 2018-04-25 (FLOW, Human)

Wu X, Li Y, Liu X et al. Targeting B7-H1 (PD-L1) sensitizes cancer cells to chemotherapy Heliyon 2018-12-01 [PMID: 30603685] (FLOW, Human)

Meunier M, Scandolera A, Chapuis E et al. From stem cells protection to skin microbiota balance: Orobanche rapum extract, a new natural strategy J Cosmet Dermatol 2018-11-28 [PMID: 30485658] (ICC/IF, Human)

Qi G, Tuncel H, Aoki E et al. Intracellular localization of survivin determines biological behavior in colorectal cancer. Oncol Rep 2009-09-01 [PMID: 19639203] (WB, IF/IHC, Human)

Hori M, Miki T, Okamoto M et al. The Detergent-Soluble Cytoplasmic Pool of Survivin Suppresses Anoikis and Its Expression Is Associated with Metastatic Disease of Human Colon Cancer. PLoS One 2013-01-01 [PMID: 23405201] (WB, Human, Hamster)

Guvenc H, Pavlyukov MS, Joshi K et al. Impairment of Glioma Stem Cell Survival and Growth by a Novel Inhibitor for Survivin-Ran Protein Complex. Clin Cancer Res 2013-01-18 [PMID: 23251006] (IP, Human)

Lechler P, Wu X, Bernhardt W et al. The tumor gene survivin is highly expressed in adult renal tubular cells: implications for a pathophysiological role in the kidney. Am J Pathol171(5):1483-98. 2007-11-01 [PMID: 17982126] (IHC-P)

Lechler P, Renkawitz T, Campean V et al. The antiapoptotic gene survivin is highly expressed in human chondrosarcoma and promotes drug resistance in chondrosarcoma cells in vitro. BMC Cancer11(1):120. 2011-04-02 [PMID: 21457573]

Ding Y, Prieto VG, Zhang PS, Rosenthal S, Smith KJ, Skelton HG, Diwan AH. Nuclear expression of the antiapoptotic protein survivin in malignant melanoma. Cancer106(5):1123-9. 2006-03-01 [PMID: 16456815] (IHC-P, Human)

Giodini, A et al. Regulation of microtubule stability and mitotic progression by survivin. Cancer Res62(9):2462-7. 2002 -05-01 [PMID: 11980633]

Fortugno P, Wall NR, Giodini A, O'Connor DS, Plescia J, Padgett KM, Tognin S, Marchisio PC, Altieri DC. Survivin exists in immunochemically distinct subcellular pools and is involved in spindle microtubule function. J Cell Sci115(Pt 3):575-85. 2002-02-01 [PMID: 11861764] (WB, IP, ELISA, ICC/IF, Human)

Lechler P, Schaumburger J, Kock FX et al. The Oncofetal Gene Survivin Promotes Cell Proliferation and Survival in Primary Human Osteoblastic Cells. Calcif Tissue Int. 2011-06-15 [PMID: 21674243] (ICC/IF, Human)



#### **Procedures**

### Serum protocol for Survivin Antibody (NB500-237)

Survivin Antibody (32.1):

IHC-FFPE sections

- I. Deparaffinization:
- A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
- B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
- II. Quench Endogenous Peroxidase:
- A. Place slides in peroxidase quenching solution: 15-30 minutes. To Prepare 200 ml of Quenching Solution: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.

  Use within 4 hours of preparation
- B. Place slides in distilled water: 2 changes for 2 minutes each.
- III. Retrieve Epitopes:
- A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celsius.
- B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
- C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
- D. Slowly add distilled water to further cool for 5 minutes.
- E. Rinse slides with distilled water. 2 changes for 2 minutes each.
- IV. Immunostaining Procedure:
- A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
- B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
- C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
- D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
- E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
- G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.



- I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
- K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
- L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
- M. Rinse slides in distilled water.
- N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
- O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
- P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
- Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
- R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
- S. Lay slides on a flat surface to dry prior to viewing under microscope.

#### NOTES:

- -Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
- -Prior to deparaffinization, heat slides overnight in a 60 degrees Celsius oven.
- -All steps in which Xylene is used should be performed in a fume hood.
- -For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
- -For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
- -200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
- -5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
- -Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).





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## **Products Related to NB500-237**

NB800-PC9 HeLa Nuclear Cell Lysate

HAF007 Goat anti-Mouse IgG Secondary Antibody [HRP]

NB720-B Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]

NBP1-43319-0.5mg Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

#### 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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