Product Datasheet

Survivin Antibody - BSA Free NB500-201

Unit Size: 0.1 ml

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

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NB500-201

Survivin Antibody - BSA Free

Product Information			
Unit Size	0.1 ml		
Concentration	1 mg/ml		
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.		
Clonality	Polyclonal		
Preservative	0.02% Sodium Azide		
Isotype	IgG		
Purity	Immunogen affinity purified		
Buffer	PBS		
Target Molecular Weight	16 kDa		
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# 015392]		
Product Application Details			
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Dual RNAscope ISH-IHC, Knockdown Validated		
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:25, Flow Cytometry reported in scientific literature (PMID 17875988; 33737139), ELISA reported in scientific literature (PMID 24102797), Immunohistochemistry 1:50-1:100, Immunocytochemistry/ Immunofluorescence 1:50-1:250, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:50-1:500, Immunohistochemistry-Frozen reported in scientific literature (PMID 12671708), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Knockdown Validated, Dual RNAscope ISH-IHC		
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. 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. 		



Images





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).



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

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 permeablized 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.









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





Immunohistochemistry: Survivin Antibody [NB500-201] -Immunohistochemical analysis using [NB500-201]. The top photo is a

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

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] -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 [NB500-201], followed by 1:2000 dilution of goat antirabbit IgG.















Overexpression of Survivin predominantly protects anoikis. B). Caspase-3 activation in EGFP- and EGFP-Survivin-expressing cells after serum starvation under attached or detached culture conditions. The experimental protocol was illustrated in Figure S1. Transfection frequencies were checked by using fluorescence microscopy and confirmed to be 80-90%. Cells were kept in serum-free medium for 24-72 h, harvested, and lysed in Laemmli SDS-sample buffer for immunoblot analysis with anti-GFP, anti-Survivin, anti-activated caspase-3 antibody, anti-LC3B, and anti-alpha-tubulin. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0055710), licensed under a CC-BY licence. Survivin regulates autophagic flux. (A) U2OS cells stably expressing Α survivinWTGFP or GFP alone were treated with RAP (200 nM) and CQ kDa (120 uM) for 2 h, then lysed and immunoblotted with anti-LC3, antisurvivin and anti-tubulin antibodies. Immunoblot shown is representative 17 of four independent experiments. Image collected and cropped by CiteAb from the following publication

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(-) or 2 uM lapatinib (+) in lapatinib-resistant cells (A), cells

following publication

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Short term nicotine exposure does not alter Lypd6, Lynx1, or Ly6H levels in the brain. Lypd6, Lynx1, Ly6H, and β 2 nAChR subunit protein levels were analyzed in (a) frontal cortex (FC) and hippocampal (HIP) tissue from rats administered nicotine (0.4 mg/kg s.c., twice daily) or vehicle (0.9% saline) for 7 days from day 8–14 or (b) 54–60 (n = 8). (c and d) Representative images of western blots summarized in (a) and (b), respectively. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/27344019), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

UroA/UAS03 enhance tight junction proteins in AhR-dependent manner. a HT29 cells were treated with vehicle (DMSO-0.01%)/UroA/UAS03 (50 µM) for 24 h. mRNA levels of Cytochrome P450 1A1 (Cyp1A1) was measured by RT PCR. b Cyp1A1 protein levels were measured using immunoblots and quantified band intensities by Image J software. c Cyp1A1 enzyme activity was measured by P450-Glo Cyp1A1 assay. HT29 cells were treated with UroA or UAS03 (0.1, 1, 10, 25, 50 µM) or FICZ (0.1, 1, 10, 25, 50 nM) for 24 h and enzyme Cyp1A1 activity was measured. d C57BL/6 and AhR-/- (n = 3) mice were treated orally with Vehicle (0.25% CMC), UroA or UAS03 (20 mg/kg) for 1 week and Cvp1A1 activity was measured in colons and livers by ethoxyresorufin-Odeethylase (EROD) assay. e The cells expressing AhR-reporter (luciferase) were treated with Veh or UroA/UAS03 or ellagic acid (EA) or MeBio (AhR high affinity ligand) for 6 h and fold change of luminescence over vehicle treatment was measured. f Immunofluorescence confocal images of HT29 cells treated with vehicle/UroA/UAS03 (50 µM) for 6 h. The cells were stained with anti-AhR antibody (red) and DAPI (blue). Relative fluorescence (n = ~20 cells) in the cytosol and nucleus was measured. The scale bar indicates 10 µm. g AhR levels in cytosol and nuclear fractions of HT29 cells treated for 2 h with Veh or UroA/UAS03 (50 µM). h AhR or i Cyp1A1 was knocked down using siRNA in HT29 cells and the cells were treated with vehicle/UroA/UAS03 (50 μ M) for 24 h and immnunoblots were performed to detect expression of AhR, Cyp1A1, and Cldn4. Scrambled (Sc) siRNA transfections were used as controls. Immunoblots were quantified using Image J software. The data is representative of two independent repeats with triplicate wells for each treatment. Statistics performed using unpaired t-test using Graphpad Prism software. All in vitro studies were performed in triplicates. Error bars, ±SEM; ***p < 0.001; **p < 0.01; **p < 0.05. Source Data are provided as a Source Data File Image collected and cropped by CiteAb from the following open publication

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hPaca1

actin

		gem	-	+		+
	ctrl miR-203	cleaved				-
survivin	-	caspase-3				-
actin		tubulin	-	-	-	

tubulin

miR-203

ctrl



Characterization of MSCmix and NCSCmix isolated from the bone marrow of adult Wnt1-CRE/R26R-LacZ mice. After recombination, NCSCs from Wnt1-CRE/R26R-LacZ mice express LacZ gene. MSCs did not undergo Cre/Lox recombination and conserved the PGK-Neo cassette (a). MSCmix are adherent fibroblast-like cells, do not express β -galactosidase (b) or Sox2 c (red), slightly express Nestin (c) (green), p75NTR (d) (red), and Sca-1 (d) (green). NCSCmix have a similar morphology, express β -galactosidase (e), Nestin (f) (green), Sox2 (f) (red) and p75NTR (g) red), but not Sca-1(g) (green). Scale bar = 20 µm. MSC mesenchymal stem cell, NCSC neural crest stem cell Image collected and cropped by CiteAb from the following open publication (https://stemcellres.biomedcentral.com/articles/10.1186/s13287-015-0202-2), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Aryl hydrocarbon receptor interacting protein (AIP) immunostaining.A and B—Examples of low AIP expression; C and D: Examples of high AIP expression; E—Normal human pituitary staining with omitting primary antibody (negative control); F—Normal human pituitary staining with AIP (positive control); Scale bar = 1000 μ m. Image collected and cropped by CiteAb from the following open publication

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Repression of p21 by APE1 in p53-null cells and effect of ectopic p53 in this repression.(A & B) Real Time RT-PCR analysis showing relative quantitation of p21 transcript level in (A) HCT116p53null cells with WT and N Δ 42 APE1 overexpression; *: p value (n=4) calculated from control (empty vector transfection) vs. WT or N Δ 42 APE1 overexpression, and (B) control (control siRNA) vs. APE1-depleted HCT116p53null cells; *: p value <0.05 (n=4) calculated from control vs. APE1-depleted cells. (C) Effect of ectopic p53 expression on p21 transcript level in control vs. APE1-depleted HCT116p53null cells. First, cells were transfected with control siRNA or APE1 siRNA, the next day both the cell types were again transfected with empty vector or p53 expression vector and after 48 hrs the cells were harvested; signal from empty vector transfection in both control and APE1-depleted cells were set as reference samples; *: p value <0.05 (n=3) calculated based on the effect of ectopic p53 expression over empty vector transfection in control vs. APE1-depleted cells. (D) Effect of APE1 depletion in control (empty vector transfected) vs. ectopic p53-expressing HCT116p53null cells; the same experiment was performed as in C but analyzed differently; signal from control siRNA-transfected cells in both empty vector transfected and ectopic p53 expressing cases were set as reference samples; *: p value <0.05 (n=3) calculated based on the effect of APE1-depletion in empty vector transfected vs. ectopic p53 expressing cells. (E) Representative Western analysis of p53, APE1, p21 and α -Tubulin levels in the same HCT116p53null cells as in B–D. (F & G) Real Time RT-PCR analysis of p21 level in Saos2 cells as in C & D. *: p value <0.05 (n=2). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/23874636), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





Pre-treatment with 0.5 mM sodium arsenite (SA) enhances permissivity in a cell-type-specific manner across reovirus strains. (A) CV-1, HeLa, L929, or HPDE cells were left untreated (no SA) or were treated with 0.5 mM SA for 30 min prior to infection (Pre-SA). Following this, cells were infected with T3D such that ~20% to 50% of cells were infected and at 18 h p.i. cells were fixed and immunostained for µNS and DAPI to visualize viral factories (VFs). The percent of cells containing VFs was quantified ((# of cells containing VFs/total # of cells) × 100) from three independent experiments. The expression level of μ NS (B) and μ 1 (C) was determined in CV-1, L929, or HeLa cells either left untreated (no SA) or treated with 0.5 mM SA for 30 min (Pre-SA) before infection with T3D at MOI = 1. At 18 h p.i., cells were harvested and the expression level of the indicated proteins was determined by immunoblot. M = mock. Densitometry analysis of the band intensity for µNS and µ1 was adjusted to the matched α-tubulin loading control for two independent experiments. Columns represent mean ± SEM. (D) CV-1; (E) L929; or (F) HeLa cells were left untreated (no SA) or were treated with 0.5 mM SA prior to infection (Pre-SA). Cells were then infected with the reovirus strains, T3D, T1L, or T3A, as described in (A). At 18 h p.i., cells were fixed and immunostained for µNS and DAPI to detect VFs. The percent of cells containing VFs was quantified ((# of cells containing VFs/total # of cells) \times 100) from at least two independent experiments. * p < 0.05: ** p < 0.01; two-tailed unpaired t test. The error bars indicate S.D. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31216693), licensed under a CC-BY license. Not internally tested by Novus Biologicals. Inhibition of Rac1 by N17Rac1 mutant or Rac1 siRNA diminishes IRinduced G2/M checkpoint activation. (A) MCF-7 cells were infected with Ad.N17Rac1 or Ad.Control for 24 hours and exposed to 15-Gy IR. Left panel: the cells were analyzed for DNA content 24 hours after IR. The result depicts the percentage of cells with 4N-DNA content and is shown as mean \pm SD of quadruplicate samples. *P < 0.001 (n = 4), significant difference from the irradiated Ad.Control-infected cells. Right panel: Inset: at 15 minutes after IR, the infected cells were analyzed for Rac1 activities (Rac1-GTP) and protein levels (total Rac1). Bar graph: mitotic cells in the cell samples were analyzed 2 hours after IR. The result depicts the percentage of mitotic cells and is shown as mean ± SD of triplicate samples. **P = 0.002 (n = 3), significant difference from the irradiated Ad.Control-infected cells. (B) Upper panel: MCF-7 cells transfected with Rac1 siRNA (Rac1) or control siRNA (Control) were incubated for the indicated times and analyzed for protein levels of Rac1 and Actin. Lower panel: After 2-day incubation, the siRNA-transfected cells were exposed to IR, incubated for 24 hours, and assessed for DNA content. Results depict the percentage of cells with 4N-DNA content and represent the mean \pm SD of three separate experiments in duplicate samples. *P < 0.001 (n = 6), significant difference from the irradiated Control-siRNA transfected cells. (C) After 2-day incubation, siRNA-

transfected cells were treated with/without 20-Gy IR, incubated for 1 hour, and analyzed for ATM, ATR, Chk1, and Chk2 activities. Image collected and cropped by CiteAb from the following open publication (http://breast-cancer-

research.biomedcentral.com/articles/10.1186/bcr3164), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





Myocardial infarction (MI) increased TLR3 expression and its physical association with Trif in mouse myocardium. Heart samples were taken from infarct area at 4 weeks after MI. (A) and (B) show mRNA and protein levels of TLR3 in sham and MI hearts. n = 4 mice/group. Data are means ± S.D. AP < 0.01 versus sham. (C) Representative immunohistochemistry images of heart sections stained for TLR3 (brown colour). An isotype IgG control was performed to verify the specificity of TLR3 reactivity. (D) Lysates of heart tissue were immunoprecipitated with anti TLR3 antibodies (IP: TLR3), followed by SDS-PAGE and immunoblotting (IB) with indicated antibodies. IP with isotype IgG (IP: IgG) was performed as a control to exclude the non specific binding of antibodies to cellular proteins. Green arrows indicate non specific bands. The association between TLR3 and Trif, but not MyD88, was detectable in sham myocardium and was increased in infarct myocardium. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/28945004), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Vps13 co-fractionates with Rab7 and Rab5.(A) Western blot analysis of control fly head samples fractionated into a cytosolic and membrane fraction from postnuclear supernatant (PNS). EGFR was used as a membrane marker and GAPDH as a cytosolic marker. (B) Membrane fractions from control fly heads treated with 1 M KCI, Na2CO3 pH 11 or 6 M urea were centrifuged to separate the soluble and insoluble (membrane containing) fractions. The level of Vps13 was determined in these fractions. Markers for peripheral membrane proteins (GM130), integral membrane proteins (EGFR) and the cytosolic proteins (GAPDH) were used. The "Vps13 lysate" lane contains a lysate derived from Vps13 homozygous mutant fly heads, as expected no Vps13 is detected, demonstrating the specificity of the antibody against Vps13. (C) Membranes from control fly heads were fractionated on a sucrose gradient. Western blot analysis was performed to analyze the distribution of Vps13 in relation to markers associated with membranes of various organelles: Rab7 (late endosomes), Rab5 (early endosomes), GM130 (golgi), Lamp1 (lysosomes) and ATP5A (mitochondria). (D) Immunoisolation of membranes from fraction 14 of the sucrose gradient using Vps13 NT, Rab7 and Rab5 antibodies. (E) Quantification of the sucrose gradient fractionation of Fig 2C. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/28107480), licensed under a CC-BY

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Publications

Krajnik A, Nimmer E, Brazzo JA et al. Survivin regulates intracellular stiffness and extracellular matrix production in vascular smooth muscle cells APL bioengineering 2023-12-01 [PMID: 37868708] (WB, Human)

Rice K Survivin Is a Mediator of Stiffness-Induced Migration of Vascular Smooth Muscle Cell Thesis 2023-01-01 (ICC/IF, Human)

Details:

1:100 dilution

Breder-Bonk C, Docter D, Barz M et al. The Apoptosis Inhibitor Protein Survivin Is a Critical Cytoprotective Resistor against Silica-Based Nanotoxicity Nanomaterials (Basel, Switzerland) 2023-09-12 [PMID: 37764575] (IHC, Mouse, Human)

Dyshlovoy SA, Busenbender T, Hauschild J et al. Cytotoxic N-Methylpretrichodermamide B Reveals Anticancer Activity and Inhibits P-Glycoprotein in Drug-Resistant Prostate Cancer Cells Marine Drugs 2022-09-23 [PMID: 36286421] (B/N)

Drpic Danica, Almeida Ana C, Aguiar Paulo et al. Chromosome Segregation Is Biased by Kinetochore Size. Current Biology : Cb 2018-01-01 [PMID: 29706521] (B/N)

Krieg S, Roderburg C, Fung S et al. Nuclear survivin is a prognosticator in gastroenteropancreatic neuroendocrine neoplasms: a meta-analysis Journal of Cancer Research and Clinical Oncology 2022-09-01 [PMID: 35428913] (IHC)

Dyshlovoy SA, Pelageev DN, Jakob LS et al. Activity of New Synthetic (2-Chloroethylthio)-1,4-naphthoquinones in Prostate Cancer Cells Pharmaceuticals (Basel) 2021-09-22 [PMID: 34681173]

Fuller RN, Kabagwira J, Vallejos PA et al. Survivin Splice Variant 2? Enhances Pancreatic Ductal Adenocarcinoma Resistance to Gemcitabine OncoTargets and Therapy 2022-10-10 [PMID: 36238134] (WB)

Kwon M, Park J, Ko E et al. Ibulocydine inhibits migration and invasion of TNBC cells via MMP-9 regulation Research Square 2023-07-07 (WB, Human)

Ryan M Investigating Induction of DNA Damage and Angiogenic Processes as Therapeutic Targets in the Treatment of Anaplastic Thyroid Cancer Thesis 2023-01-01

Stahl P, Kollenda S, Sager J et al. Tuning Nanobodies' Bioactivity: Coupling to Ultrasmall Gold Nanoparticles Allows the Intracellular Interference with Survivin Small (Weinheim an der Bergstrasse, Germany) 2023-04-10 [PMID: 37035950] (ICC/IF, PLA, WB, Human)

Dyshlovoy S, Shubina L, Makarieva T et al. New Guanidine Alkaloids Batzelladines O and P from the Marine Sponge Monanchora pulchra Induce Apoptosis and Autophagy in Prostate Cancer Cells Marine Drugs 2022-11-25 [PMID: 36547885] (WB, Human)

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



Procedures

Western Blot protocol for 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-HCI / 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:

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:

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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Products Related to NB500-201

NB800-PC1	HeLa Whole Cell Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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