Product Datasheet

Speedy/Ringo Antibody - BSA Free
NB100-2521

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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Publications: 7

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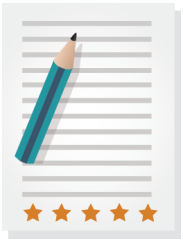
Updated 2/21/2025 v.20.1



NB100-2521

Speedy/Ringo Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7.0 - 8.0)

Product Description

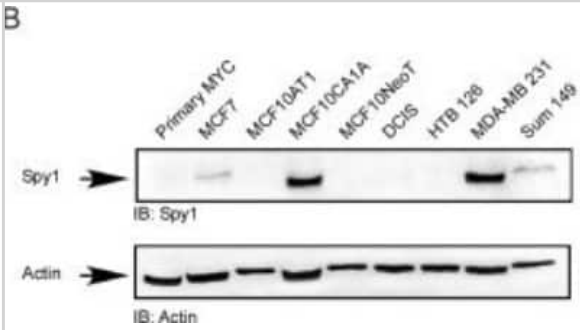
Host	Rabbit
Gene ID	245711
Gene Symbol	SPDYA
Species	Human, Mouse, Rat, Sheep
Reactivity Notes	Sheep reactivity reported in scientific literature (PMID: 19187565). Human reactivity reported in scientific literature (PMID: 24037419). Rat reactivity reported in scientific literature (PMID: 19075091).
Immunogen	A synthetic peptide made to a C-terminal region of human Speedy/Ringo (within residues 200-286). This immunogen is conserved in both isoforms 1 and 2. [Swiss-Prot Q5MJ69]

Product Application Details

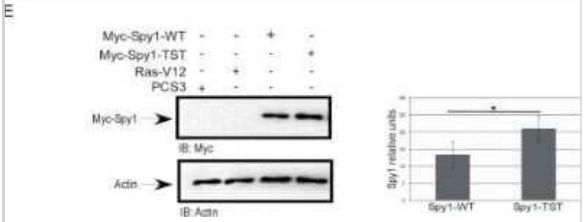
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 2 ug/ml, Immunohistochemistry reported in scientific literature (PMID 19187565), Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 19187565), Immunohistochemistry-Paraffin
Application Notes	In Western blot analysis, a band is seen at ~36 kDa (isoform 2). After a longer exposure, a 31 kDa band can be detected (isoform 1). There is also a non-specific band at ~58 kDa.

Images

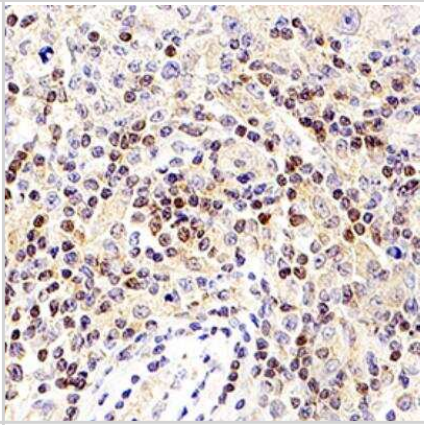
Western Blot: Speedy/Ringo Antibody [NB100-2521] - Spy1 protein levels are elevated in human breast cancers. TMAs containing cores from invasive ductal carcinoma (IvDC), infiltrated ductal carcinoma (IfDC), intraductal carcinoma (IDC) and invasive lobular carcinoma (ILC) as well as pair-matched normal (PM-N) or cancer-free patients (CF-N) were analyzed for Spy1 expression. The Spy1 signal intensity was normalized to nuclear stain (TOTO-3/PI) signal. Patient numbers are indicated below the sample (N). Breast normal and cancer cell lines were analyzed by western blot analysis. Actin was used as a loading control. One representative blot of 2. Image collected and cropped by Citeab from the following publication (The cyclin-like protein Spy1/RINGO promotes mammary transformation and is elevated in human breast cancer. *BMC Cancer* (2012)) licensed under a CC-BY license.



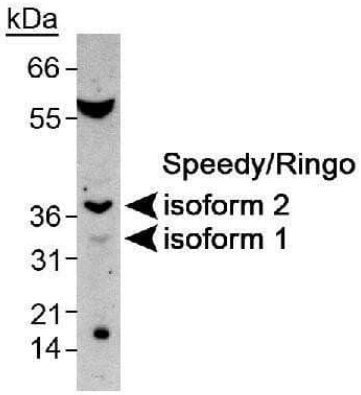
Western Blot: Speedy/Ringo Antibody [NB100-2521] - Spy1 stable protein induces anchorage independent growth. Representative foci after 14 day soft agar assay visualized using light microscopy. Ras-V12 is transfected as a positive control. n = 3. Total numbers of foci were counted over 3 separate plates using separate transfections for each experiment. n = 3. Error bars reflect SE between triplicate experiments. t test was performed. Western blot analysis of experiments performed. Quantified using densitometry followed by normalization for Actin levels. Image collected and cropped by Citeab from the following publication (The cyclin-like protein Spy1/RINGO promotes mammary transformation and is elevated in human breast cancer. BMC Cancer (2012)) licensed under a CC-BY license.



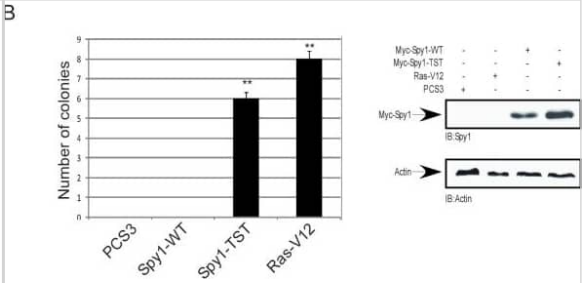
Immunohistochemistry-Paraffin: Speedy/Ringo Antibody [NB100-2521] - Speedy/Ringo was detected in immersion fixed paraffin-embedded sections of human testis using Rabbit Anti-Human Speedy/Ringo polyclonal Antibody (Catalog # NB100-2521) at 1:300 for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the nuclei of sperm cells.

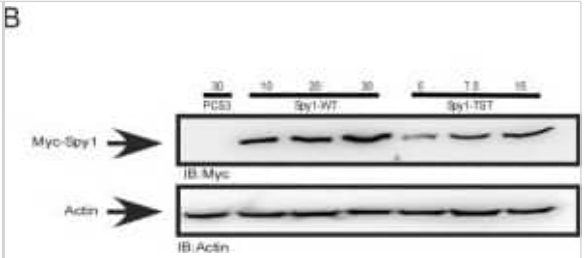
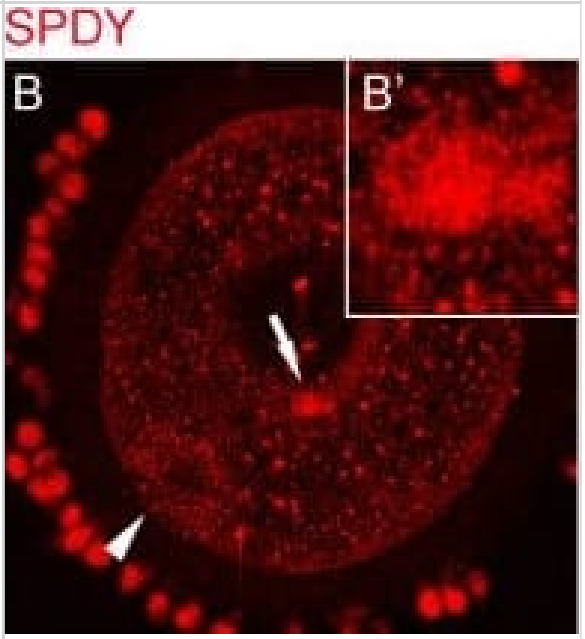


Western Blot: Speedy/Ringo Antibody [NB100-2521] - Detection of Speedy/Ringo in mouse testis (2ug/ml).

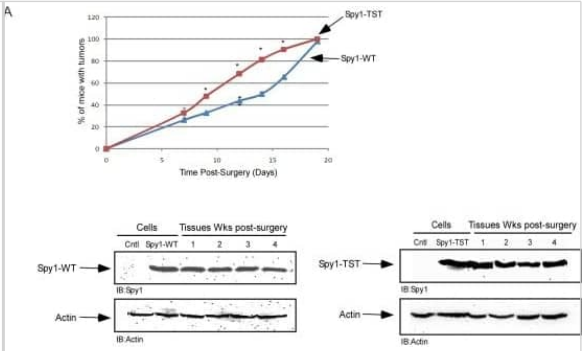


Western Blot: Speedy/Ringo Antibody [NB100-2521] - Elevated Spy1 levels prevent contact inhibition. (A) Representative views of focus formation assays in NIH3T3 cells transfected with Myc-Spy1-WT, Myc-Spy1-TST, positive control Ras-V12 or empty vector PCS3 control. n = 3 (B) Quantification of the number of colonies. Error bars reflect SE between triplicate experiments. t test was performed;** P ≤ 0.01. n = 3 (right panel) Western blot analysis of one representative lysate from A-B. Image collected & cropped by CiteAb from the following publication (https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-12-45), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

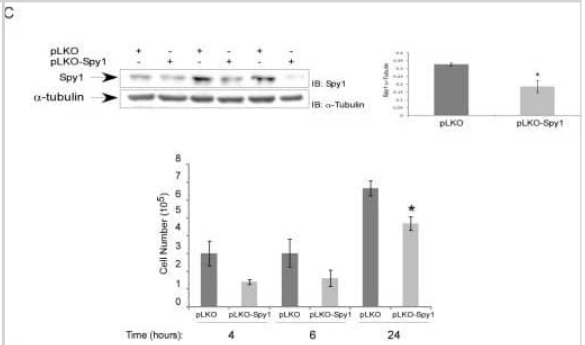


Western Blot: Speedy/Ringo Antibody [NB100-2521] - Spy1 stable protein induces anchorage independent growth. (A) 293 cells were transfected with different concentrations of Myc-Spy1-WT, Myc-Spy1-TST or 30 µg of empty PCS3 vector control. Soft agar assay was carried out & plates photographed & quantified on day 14. Foci were averaged over 3 separate transfections for each experiment. n = 3 (B) Western blot analysis of one representative experiment; densitometry in lower panel. n = 3 (C) Representative foci after 14 day soft agar assay visualized using light microscopy. Ras-V12 is transfected as a positive control. n = 3. (D) Total numbers of foci were counted over 3 separate plates using separate transfections for each experiment. n = 3. Error bars reflect SE between triplicate experiments. t test was performed;** P ≤ 0.01. (E) Western blot analysis of experiments in Figure. 1C & D. Quantified using densitometry followed by normalization for Actin levels. (A-E) Error bars reflect SE between triplicate experiments. t test was performed;* P≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001. Image collected & cropped by CiteAb from the following publication (https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-12-45), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: Speedy/Ringo Antibody [NB100-2521] - CDC2 associates with SPDY at ERES. (A-C) 0 h GV stage oocyte labeled for CDC2 (A, green), DNA (A, blue), & SPDY (B, red). Both CDC2 & SPDY localize to the same cortical domain (C). Note that the center of this oocyte is dented (the area that contains the arrow) causing the structure to appear in the middle of the oocyte, whereas it is located in the cortex. (D-F) 0 h GV stage oocyte stained for Cyclin B (D, green), DNA (D, blue), & P-GM130 (E, red). Cyclin B did not localize to the P-GM130-labeled cortical domain (F). (G-I) 0 h GV stage oocyte labeled for PSTAIR (G, green), DNA (G, blue), & P-GM130 (H, red). Spatial overlap of PSTAIR & phosphorylated GM130 staining (yellow) in a cortical domain is evident in the merged image (I). Images are Z-projections of 2 (A-C) or 3 (D-I) consecutive sections; scale bars represent 20 µm in C, F, & I, & 5 µm in C', F', & I'. Arrows indicate the region of the oocyte that is shown enlarged in the insets (A'-I'). Arrowheads denote the position of the GV. Image collected & cropped by CiteAb from the following publication (https://bmcdevbiol.biomedcentral.com/articles/10.1186/1471-213X-9-8), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: Speedy/Ringo Antibody [NB100-2521] - Spy1 stable protein accelerates tumorigenesis in vivo.(A) Percentage of mice presenting with palpable tumors from 0-19 days post-transplant. Each data point represents 4 mice per indicated construct. The entire experiment was repeated three times using three independently derived overexpressing cell lines for each construct. Mann-Whitney Test was performed ($p < 0.05$). (A; lower blot) Western blots were conducted to measure the stability of Myc-Spy1-WT (left) & Myc-Spy1-TST (right) from representative time points. Empty vector control (Cntl) cell expression levels of Spy1 are seen in lane 1 of each blot. (B) Total tumor volume was calculated for both Spy1-HC11 (Spy1-WT) & Spy1-TST-HC11 (Spy1-TST) transplanted glands. Results were taken from 45 transplants using cells from 3 separate transfections. Error bars reflect SE between transplants from different transfections. Left hand panel reflects overall volume, right hand panel reflects volumes of tissues taken over a month post-transplant. Image collected & cropped by CiteAb from the following publication (https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-12-45), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Speedy/Ringo Antibody [NB100-2521] - Spy1 protein levels are elevated in human breast cancers.(A) TMAs containing cores from invasive ductal carcinoma (IvDC), infiltrated ductal carcinoma (IfDC), intraductal carcinoma (IDC) & invasive lobular carcinoma (ILC) as well as pair-matched normal (PM-N) or cancer-free patients (CF-N) were analyzed for Spy1 expression. The Spy1 signal intensity was normalized to nuclear stain (TOTO-3/PI) signal. Patient numbers are indicated below the sample (N). (B) Breast normal & cancer cell lines were analyzed by western blot analysis. Actin was used as a loading control. One representative blot of 2. (C) Western blot of 3 replicate infections of pLKO control or pLKO Spy1 in MDA-231 cells. Middle panel represents the densitometry values over 3 separate experiments. Where Spy1 is corrected for actin levels. Lower panel reflects cell counts at 4, 6, & 24 h post-infection. (All panels) Data shown is mean ± s.d. Student's t-test was performed * $P < 0.05$;**$P < 0.01$. (D) Knockdown effects on cell counts of MCF7 cells over 72 h after transfection with either pSUPER empty vector (siCntl) or pSUPER-Spy1 (siSpy1) over 3 separate transfections. Data shown is mean ± s.d. Image collected & cropped by CiteAb from the following publication (https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-12-45), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Wang X, Zhu M, Shan D et al. Spy1, a Unique Cell Cycle Regulator, Alters Viability in ALS Motor Neurons and Cell Lines in Response to Mutant SOD1-Induced DNA Damage DNA Repair 2018-12-01 [PMID: 30630676] (WB)

Ng LF, Chow A, Sun YJ et al. IL-1beta, IL-6, and RANTES as biomarkers of Chikungunya severity. PLoS One 2009-01-01 [PMID: 19156204]

Giachini FR, Chiao CW, Carneiro FS et al. Increased activation of stromal interaction molecule-1/Orai-1 in aorta from hypertensive rats: a novel insight into vascular dysfunction. Hypertension 2009-02-01 [PMID: 19075091] (Rat)

Fei M, Hang Q, Hou S, Ruan C. Cell adhesion to fibronectin down-regulates the expression of Spy1 and contributes to drug resistance in multiple myeloma cells. Int J Hematol. 2013-10-01 [PMID: 24037419] (WB, Human)

Holzenspies JJ, Stoorvogel W, Colenbrander B et al. CDC2/SPDY transiently associates with endoplasmic reticulum exit sites during oocyte maturation. BMC Dev Biol 2009-02-03 [PMID: 19187565] (IF/IHC, ICC/IF, Sheep)

Al Sorkhy M, Ferraiuolo R-M, Jalili E et al. The cyclin-like protein Spy1/RINGO promotes mammary transformation and is elevated in human breast cancer. BMC Cancer 2612(1):45. 2012-01-01 [PMID: 22280365]

Al Sorkhy M et al. The cyclin-dependent kinase activator, Spy1A, is targeted for degradation by the ubiquitin ligase NEDD4. J Biol Chem;284(5):2617-27. 2009-01-30 [PMID: 19054764]

Procedures

Protocol specific for Speedy / Ringo Antibody (NB100-2521)
Speedy/Ringo Antibody:
Western Blot Protocol

1. Perform SDS-PAGE (4-12%, Bis-Tris) on samples to be analyzed, loading 40 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% non-fat dry milk + 1% BSA in TBS, overnight at 4 degrees Celsius.

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 rabbit anti-speedy/ringo primary antibody (NB 100-2521) 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 rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's 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 manufacturer's instructions (Pierce's 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.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
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.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NB100-2521

NB820-59672	Mouse Testis Whole Tissue Lysate (Adult Whole Normal)
NB100-2521PEP	Speedy/Ringo Antibody Blocking Peptide
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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