

Product Datasheet

CENPF Antibody - BSA Free

NB500-101

Unit Size: 0.1 mg

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

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NB500-101

CENPF Antibody - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

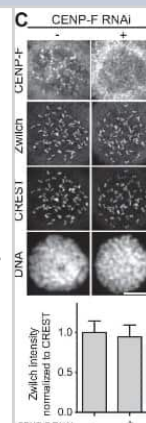
Product Description	
Description	Novus Biologicals Rabbit CENPF Antibody - BSA Free (NB500-101) is a polyclonal antibody validated for use in IHC, WB, Flow, ICC/IF and IP. Anti-CENPF Antibody: Cited in 58 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	1063
Gene Symbol	CENPF
Species	Human, Mouse, Bovine, Equine
Immunogen	A bacterial fusion protein from the C-terminus of human CENPF. [UniProt# P49454]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, Immunohistochemistry Whole-Mount, Knockdown Validated
Recommended Dilutions	Western Blot 1:1500-1:2500, Flow Cytometry 1:50-1:200, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence 1:200-1:1000immunoprecipitation 1:50, Immunoprecipitation 1:50, Immunohistochemistry-Paraffin reported in scientific literature (PMID 20398247), Immunohistochemistry-Frozen reported in scientific literature (PMID 26195156), Immunohistochemistry Whole-Mount reported in scientific literature (PMID 24876181), Knockdown Validated
Application Notes	In Western Blot, a band can be seen at ~330 kDa. In ICC/IF, nuclear staining was observed in HeLa cells.

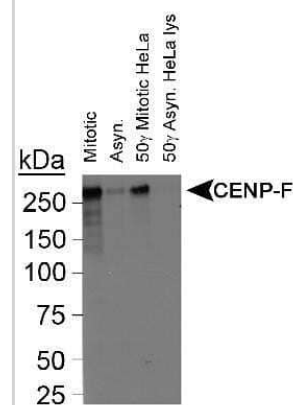


Images

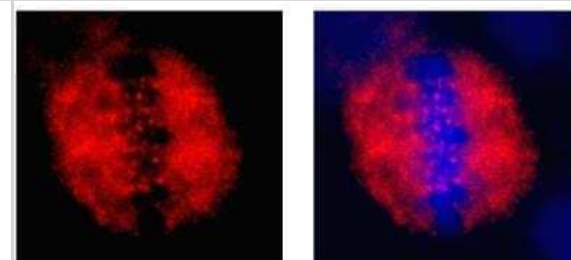
Immunocytochemistry/ Immunofluorescence: CENPF Antibody - BSA Free [NB500-101] - Kinetochores localization of RZZ and MAD1 are independent of CENP-E and CENP-F. CENP-F depletion does not interfere with the recruitment of Zwilch. The graphs show mean intensity of one (B, C, and E), two (D and F), or three (A, G, and H) experiments; the error bars indicate S.E., and the mean values for nondepleted cells are set to 1. Image collected and cropped by CiteAb from the following publication (<http://pubmed.ncbi.nlm.nih.gov/29748388/>) licensed under a CC-BY license.



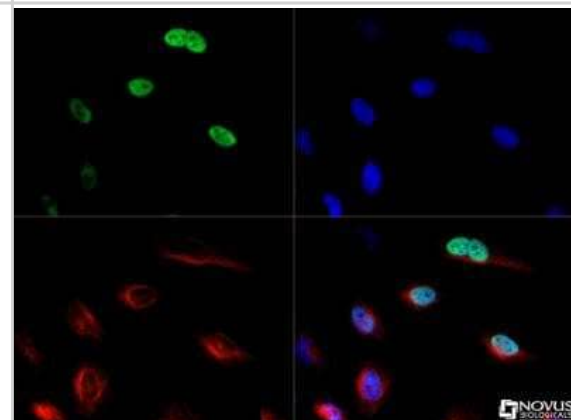
Western Blot: CENPF Antibody [NB500-101] - 25ug of asynchronous and mitotically blocked HeLa lysates were loaded in each respective lane.



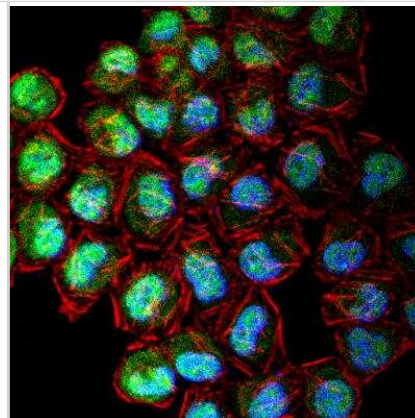
Immunocytochemistry/Immunofluorescence: CENPF Antibody [NB500-101] - Distribution of CENP-F (red), largely associated with the mitotic spindle and a clearly distinguishable frac.



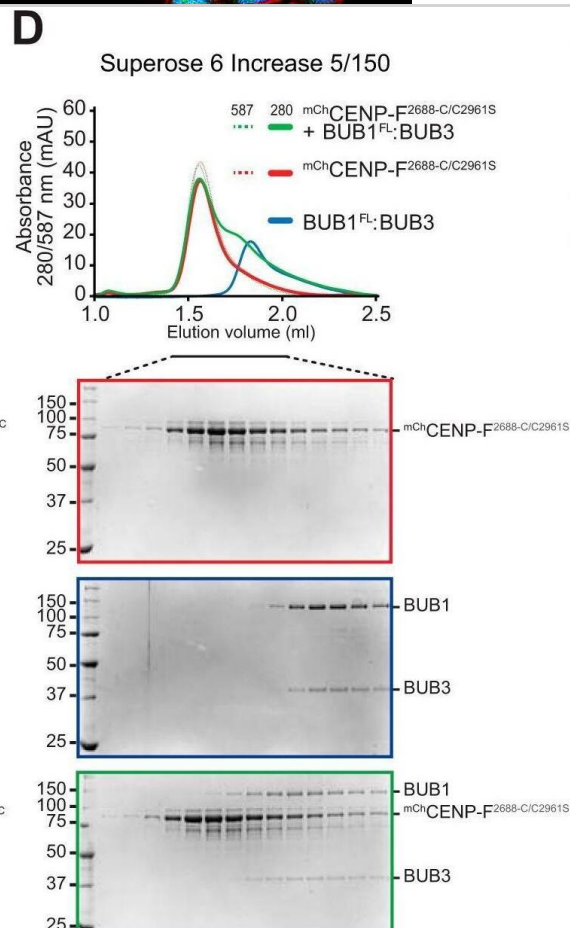
Immunocytochemistry/Immunofluorescence: CENPF Antibody [NB500-101] - CENPF antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



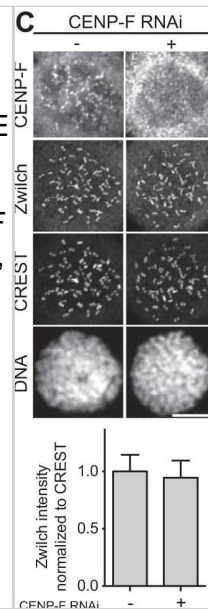
Immunocytochemistry/Immunofluorescence: CENPF Antibody [NB500-101] - Confocal immunofluorescent analysis of HeLa cells using CENPF antibody (NB500-101, 1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green, A). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red, B). DAPI was used to stain the cell nuclei (blue, C).



Requirements for CENP-F kinetochore localization. A, representative images of mitotic HeLa cells electroporated with mCherry, mCherry-CENP-F2688-C WT, or mCherry-CENP-F2688-C/C3207A (farnesylation mutant). Scale bar, 5 μ m. As for CENP-E, both the WT and the unfarnesylated mutant CENP-F constructs localize at kinetochore. B, mCherry-CENP-F2688-C sample was visualized by EM after glycerol spraying and low-angle platinum shadowing (right panel). The elongated shape of the observed particles is consistent with the secondary structure expected for the mCherry-tag coiled-coil construct (right panel). C and D, SEC elution profiles and SDS-PAGE analysis of binding experiments with 16 μ m each of mCherry-CENP-F2688-C WT (C) or C2961S mutant (D) and 4 μ m BUB1^{FL}/BUB3 complex. The shift in elution volume of BUB1/BUB3 is observed with both WT and mutant CENP-F, but it is significantly less pronounced for the CENP-F mutant, suggesting that the C2961S mutation reduces the affinity of CENP-F for the BUB1 kinase domain without completely abolishing it. E, SEC elution profile and SDS-PAGE analysis of a binding experiment with 16 μ m each of mCherry-CENP-F2688-C and eGFP-CENP-E2070-C. No shift is observed, indicating that the tested constructs do not interact. The elution profile and SDS-PAGE of eGFP-CENP-E2070-C WT in E is the same already shown in Fig. 3, B and D. Similarly, the elution profiles and SDS-PAGE of mCherry-CENP-F2688-C in E is the same as in Fig. 4, D, E, and G. Similarly, the elution profiles and SDS-PAGE of BUB1/BUB3 in C and D are the same. These repetitions were included to facilitate the interpretation of binding experiments by inclusion of elution references. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/29748388>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Kinetochores localization of RZZ and MAD1 are independent of CENP-E and CENP-F. A–H, representative images and quantification of protein kinetochores levels in HeLa cells mock-treated or depleted of Zwilch (A), CENP-E (B, E, and H), CENP-F (C, F, and G), or co-depleted of CENP-E and CENP-F (D). Scale bar, 10 μ m. Zwilch depletion does not affect the localization of CENP-E (A). CENP-E depletion does not affect the localization of Zwilch (B), MAD1 (E), and CENP-F (H). Similarly, CENP-F depletion does not interfere with the recruitment of Zwilch (C), MAD1 (F), and CENP-E (G). Co-depletion of CENP-E and CENP-F has no effects on localization of Zwilch (D). The graphs show mean intensity of one (B, C, and E), two (D and F), or three (A, G, and H) experiments; the error bars indicate S.E., and the mean values for nondepleted cells are set to 1. Elements in the left column of A (negative controls of the RNAi experiments) are also shown in Fig. S3C. Elements in G are shown again in Fig. S2E. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/29748388>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Averbek S, Jakob B, Durante M, Averbek NB. O-GlcNAcylation Affects the Pathway Choice of DNA Double-Strand Break Repair International Journal of Molecular Sciences 2021-05-27 [PMID: 34071949] (Western Blot, Human)

Luppino JM, Park DS, Nguyen SC et al. Cohesin promotes stochastic domain intermingling to ensure proper regulation of boundary-proximal genes Nature Genetics 2020-08-01 [PMID: 32572210] (Western Blot, Human)

Cmentowski V, Ciossani G, d'Amico E et al. A mechanism that integrates microtubule motors of opposite polarity at the kinetochore corona bioRxiv 2023-09-01 [PMID: 37163019] (Western Blot, Human)

Jin Zhao, Fei Wang, Qingjun Tian, Jing Dong, Liuqing Chen, Rongyi Hu Involvement of miR-214-3p/FOXO1 Axis During the Progression of Psoriasis. Inflammation 2022-03-24 [PMID: 34427853]

Sarmi Nath, Ganesh Nagaraju, Wolf-Dietrich Heyer FANCD1 helicase promotes DNA end resection by facilitating CtIP recruitment to DNA double-strand breaks PLoS Genetics 2020-04-06 [PMID: 32251466]

Cmentowski V, Ciossani G, d'Amico E et al. RZZ-Spindly and CENP-E form an integrated platform to recruit dynein to the kinetochore corona The EMBO journal 2023-11-20 [PMID: 37984321] (ICC/IF, Human)

Details:

1:300 dilution

Shi Q, Liu X, Kalashova J et al. Characterization of mitotic phenotypes associated with a MYC synthetic lethal compound bioRxiv 2023-04-06 (ICC/IF)

Cmentowski V, Ciossani G, d'Amico E et al. A mechanism that integrates microtubule motors of opposite polarity at the kinetochore corona bioRxiv 2023-04-25 (Immunocytochemistry/ Immunofluorescence, Human)

Details:

1:300 ICC/IF

Li L, Zhang X, Zhang H et al. Single-Cell and CellChat Resolution Identifies Collecting Duct Cell Subsets and Their Communications with Adjacent Cells in PKD Kidneys Cells 2022-12-22 [PMID: 36611841] (IHC-P, Mouse)

Tanaka K, Suzuki K, Miyashita K et al. Activation of recombinational repair in Ewing sarcoma cells carrying EWS-FLI1 fusion gene by chromosome translocation Scientific Reports 2022-08-30 [PMID: 36042341] (ICC/IF, Human)

Averbek NB, Barent C, Jakob B et al. The Ubiquitin Ligase RNF138 Cooperates with CtIP to Stimulate Resection of Complex DNA Double-Strand Breaks in Human G1-Phase Cells Cells 2022-08-17 [PMID: 36010636] (ICC/IF, Human)

Renda F, Magidson V, Tikhonenko I et al. Effects of malleable kinetochore morphology on measurements of intrakinetochore tension Open Biol 2020-07-01 [PMID: 32634373] (ICC/IF, Human)

More publications at <http://www.novusbio.com/NB500-101>

Procedures

Western Blot protocol for CENPF Antibody (NB500-101)

Western Blot Procedure

- 1) Resolve aliquots (30 mg) of total HeLa cell lysate on a 8.5% lo-crosslinker gel (110:1, acryl:bis) for efficient separation and transfer.
- 2) Transfer to nitrocellulose membranes in 20 mM Tris-HCL (pH 8.0)/150 mM glycine/20% (vol/vol) methanol. Transfer time for a 1 mm thick gel is 1 hour 15 minutes at 450mA.
- 3) Block membranes for 1 hour with 5% (vol/vol) nonfat dry milk/TBS-T (20 mM Tris-HCL, pH 7.6/ 137 mM NaCl/0.1% TWEEN 20)
- 4) Incubate membranes for 1 hour at room temperature (RT) in NB 500-101 diluted 1:3000 in nonfat dry milk/TBS-T.
- 5) Wash 3X30 minutes at RT with TBS-T.
- 6) Incubate membranes with alkaline phosphatase conjugated anti-rabbit IgG for 1 hour (RT).
- 7) Wash 3 x 30 minutes at RT with TBS-T
- 8) Develop with ECL reagents (Amersham) and autoradiography.





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Products Related to NB500-101

NB800-PC1	HeLa Whole Cell Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
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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