

# Product Datasheet

## CD63 Antibody (H5C6) - BSA Free NBP2-42225

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

[www.novusbio.com](http://www.novusbio.com)



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**NBP2-42225**

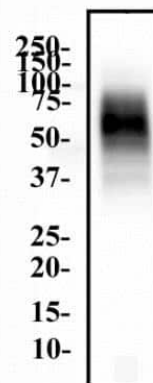
CD63 Antibody (H5C6) - BSA Free

<b>Product Information</b>	
<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	1.0 mg/ml
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	H5C6
<b>Preservative</b>	0.05% Sodium Azide
<b>Isotype</b>	IgG1 Kappa
<b>Purity</b>	Protein G purified
<b>Buffer</b>	PBS
<b>Product Description</b>	
<b>Description</b>	Novus Biologicals Mouse CD63 Antibody (H5C6) - BSA Free (NBP2-42225) is a monoclonal antibody validated for use in WB, ELISA, Flow, ICC/IF and IP. Anti-CD63 Antibody: Cited in 55 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
<b>Host</b>	Mouse
<b>Gene ID</b>	967
<b>Gene Symbol</b>	CD63
<b>Species</b>	Human, Canine
<b>Marker</b>	Exosome Marker
<b>Immunogen</b>	Human splenic adherent cells.
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Dot Blot, ELISA, Electron Microscopy, Flow Cytometry, Flow (Intracellular), Functional, Immunocytochemistry/ Immunofluorescence, In vitro assay, Immunoprecipitation, Block/Neutralize, Immunohistochemistry Whole-Mount
<b>Recommended Dilutions</b>	Western Blot, Flow Cytometry 1:1000, ELISA, Immunocytochemistry/ Immunofluorescence 1:50-1:100, Immunoprecipitation, Functional reported in scientific literature (PMID 9811687), In vitro assay reported in scientific literature (PMID 21464080), Dot Blot, Electron Microscopy reported in scientific literature (PMID 16735575), Flow (Intracellular), Immunohistochemistry Whole-Mount reported in scientific literature (PMID 21464080), Block/Neutralize reported in scientific literature (PMID 35602933)

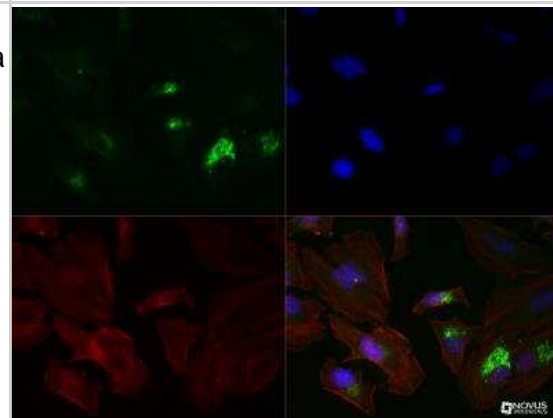


## Images

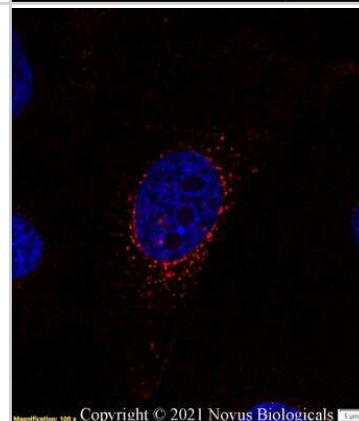
**Western Blot: CD63 Antibody (H5C6) [NBP2-42225]** - THP1 whole cell protein was separated by SDS-PAGE on a 12% gel and transferred to PVDF membrane. The membrane was probed with anti-CD63 antibody at 2 ug/mL and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



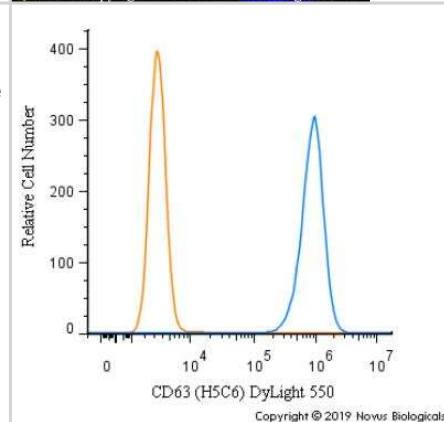
**Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225]** - The CD63 (H5C6) antibody was tested in HeLa cells at a 1:50 dilution against DyLight 488 (Green). Actin and nuclei were counterstained against Phalloidin 568 (Red) and DAPI (Blue), respectively.



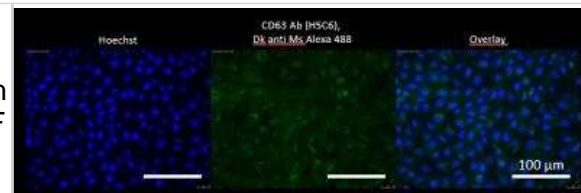
**Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225]** - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-CD63 Antibody [H5C6] conjugated to DyLight 550 (NBP2-42225R) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



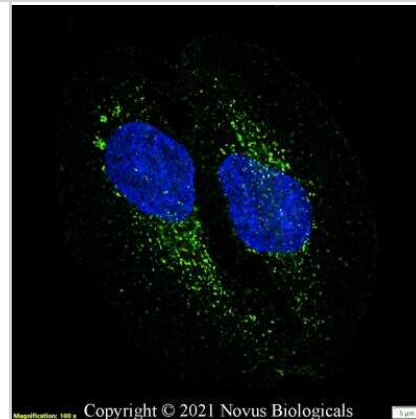
**Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225]** - An intracellular stain was performed on SK-MEL-28 cells with CD63 (H5C6) Antibody NBP2-42225R (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 550.



Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - MDCK cells stained with CD63 antibody at a dilution of 1:50 followed by Donkey anti-mouse secondary antibody conjugated with Alexa Fluor 488 (1:500). Nuclei were stained with Hoechst 33342. ICC/IF image submitted by a verified customer review.



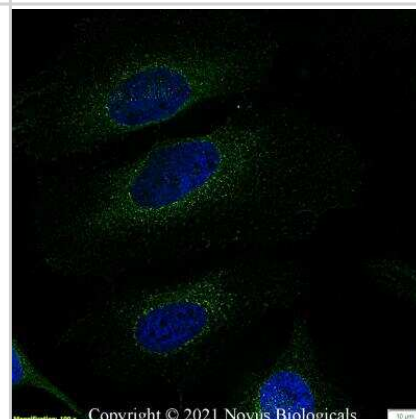
Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - U2OS cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-CD63 Antibody [H5C6] conjugated to Alexa Fluor 488 (NBP2-42225AF488) at 5 ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



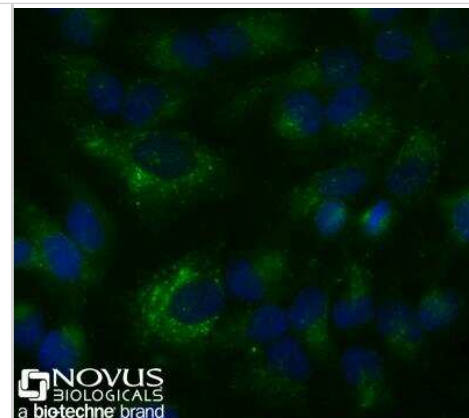
Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-CD63 Antibody [H5C6] conjugated to Biotin (NBP2-42225B) at 5 ug/ml for 60 minutes at room temperature and detected with Streptavidin Protein conjugated to DyLight 488 at a 1:500 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



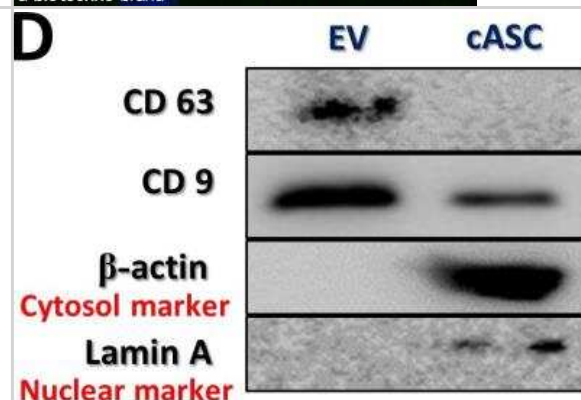
Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-CD63 Antibody [H5C6] conjugated to Biotin (NBP2-42225B) at 5 ug/ml for 1 hour at room temperature and detected with Streptavidin conjugated to DyLight 488. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



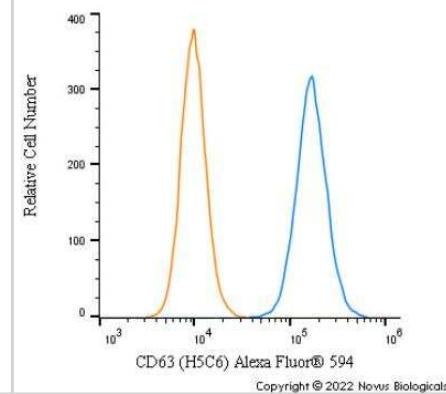
Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-CD63 [H5C6] conjugated to Alexa Fluor 488 [NBP2-42225AF488] at 10ug/mL for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



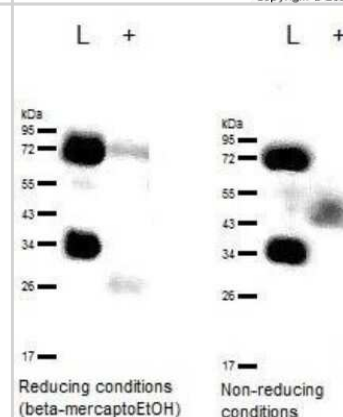
Western Blot: CD63 Antibody (H5C6) - BSA Free [NBP2-42225] - Analysis of common EV markers, where 10 ug of total protein was loaded in each lane. cASCs-EVs expressed CD63 and CD9, while beta actin and lamin A showed lower expression. The displayed data represent at least three repeated experiments with consistent results. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32040478/>) licensed under a CC-BY license.



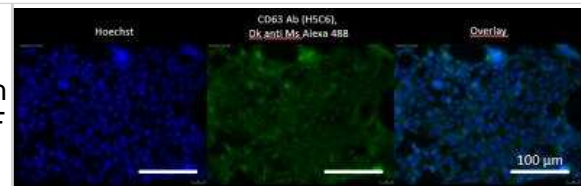
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - An intracellular stain was performed on HeLa cells with CD63 [H5C6] Antibody NBP2-42225AF594 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 594.



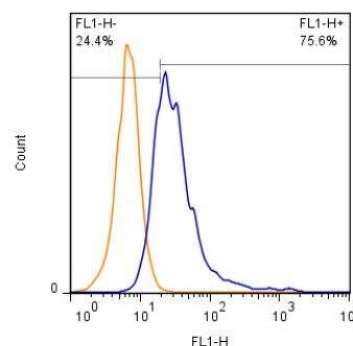
Western Blot: CD63 Antibody (H5C6) [NBP2-42225] - The same samples and volumes were run under reducing and non-reducing conditions. All procedures were performed in parallel for both conditions. L- ladder +- exosomes. Image visualized with HRP linked to secondary antibody. Western blot image submitted by a verified customer review.



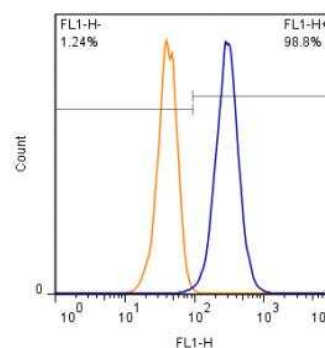
Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - HEK cells stained with CD63 antibody at a dilution of 1:50 followed by Donkey anti-mouse secondary antibody conjugated with Alexa Fluor 488 (1:500). Nuclei were stained with Hoechst 33342. ICC/IF image submitted by a verified customer review.



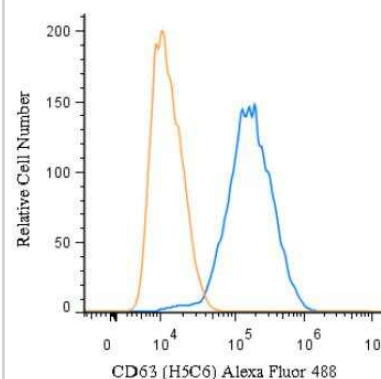
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - Human peripheral blood cells were stained ( $2 \times 10^6$  cells/mL) using the anti-CD63 antibody (Blue) at a dilution of 1:1000. Signal was detected using a Gt x Ms DyLight 488 Secondary and gated to the monocyte/granulocyte cell populations. Isotype was Mouse IgG1 kappa (orange). Data collected on BD FACS Calibur flow cytometer.



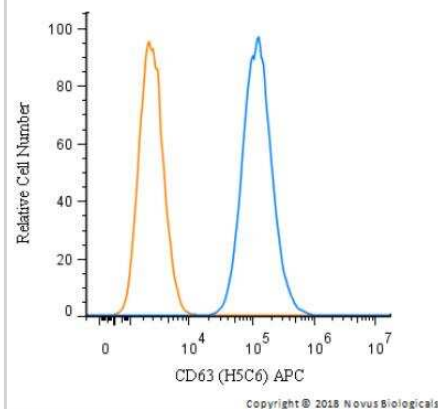
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - A431 cells were stained ( $1 \times 10^6$  cells/mL) using the anti-CD63 antibody at a 1:1000 dilution (blue). Signal was detected with Gt x Ms DyLight 488 secondary. Isotype control (orange).



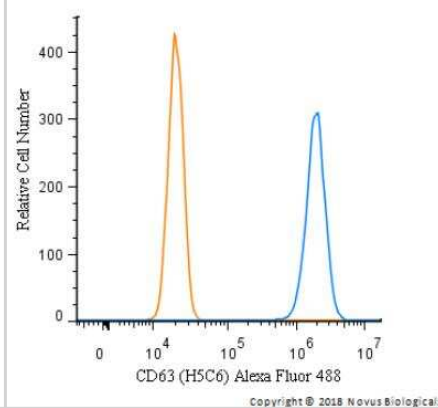
Flow (Intracellular): CD63 Antibody (H5C6) [NBP2-42225] - An intracellular stain was performed on HepG2 cells with CD63 Antibody (H5C6) NBP2-42225AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



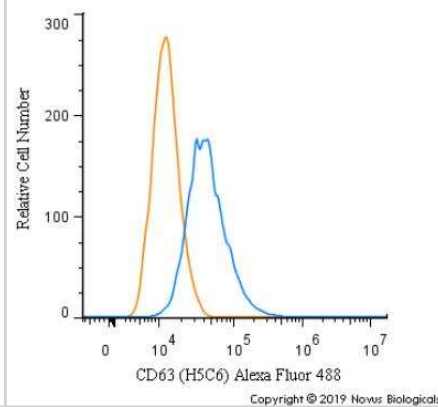
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - An intracellular stain was performed on HeLa cells with CD63 Antibody (H5C6) NBP2-42225APC (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Allophycocyanin.



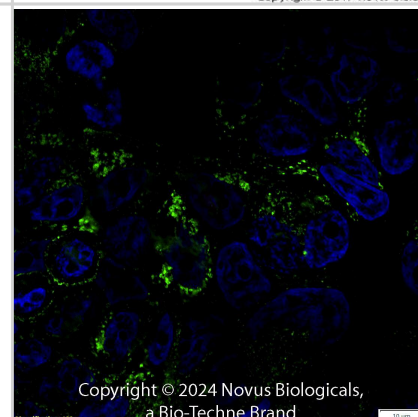
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - An intracellular stain was performed on SK-MEL-28 cells with CD63 Antibody (H5C6) NBP2-42225AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



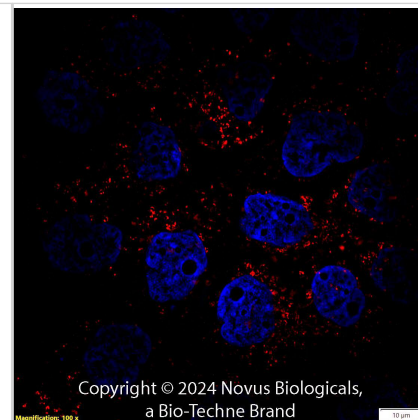
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - An intracellular stain was performed on HeLa cells with CD63 [H5C6] Antibody NBP2-42225AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



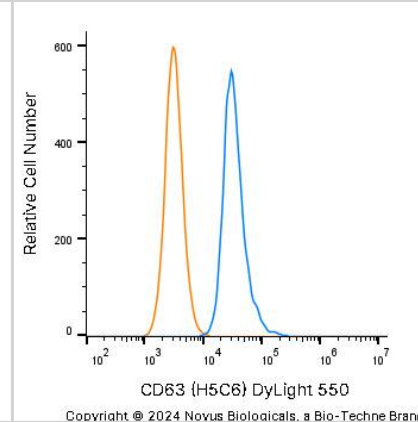
CD63 (H5C6) was detected in immersion fixed MCF7 human breast cancer cell line using Mouse anti-CD63 (H5C6) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NBP2-42225AF488) (green) at 5 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



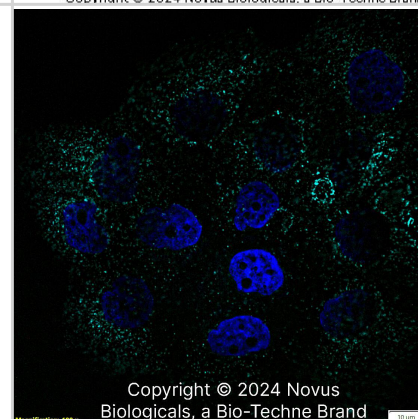
CD63 (H5C6) was detected in immersion fixed A431 human skin carcinoma cell line using Mouse anti-CD63 (H5C6) Protein-G purified Monoclonal Antibody conjugated to DyLight 550 (Catalog # NBP2-42225R) (red) at 5  $\mu\text{g}/\text{mL}$  overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



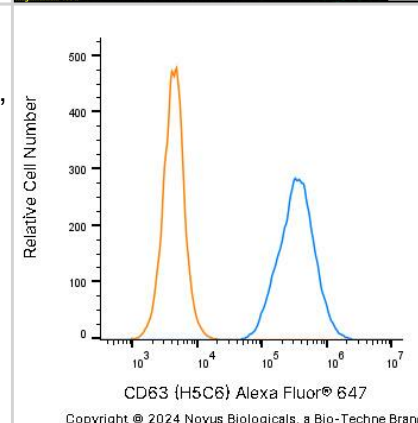
An intracellular stain was performed on A431 human skin carcinoma cell line using Mouse anti- CD63 (H5C6) Protein-G purified Monoclonal Antibody conjugated to DyLight 550 (Catalog # NBP2-42225R, blue histogram) or matched control antibody (orange histogram) at 2.5  $\mu\text{g}/\text{mL}$  for 30 minutes at RT.



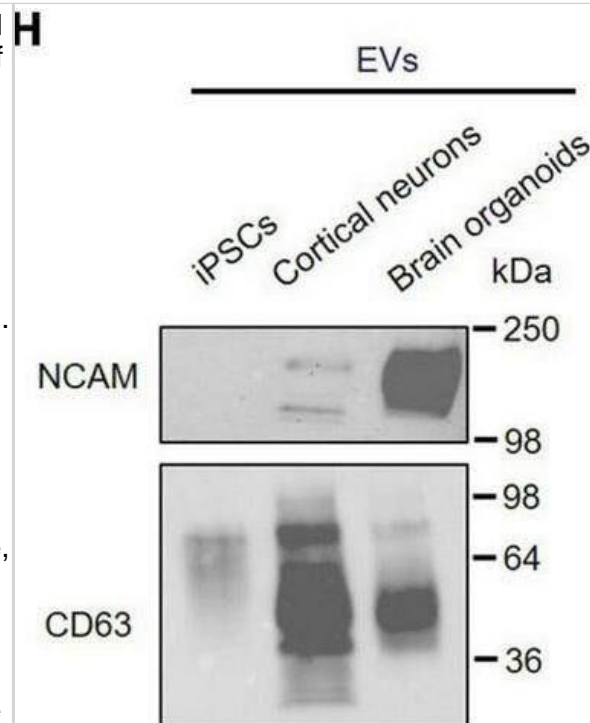
CD63 (H5C6) was detected in immersion fixed A431 human skin carcinoma cell line using Mouse anti-CD63 (H5C6) Protein-G purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP2-42225AF647) (light blue) at 2  $\mu\text{g}/\text{mL}$  overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



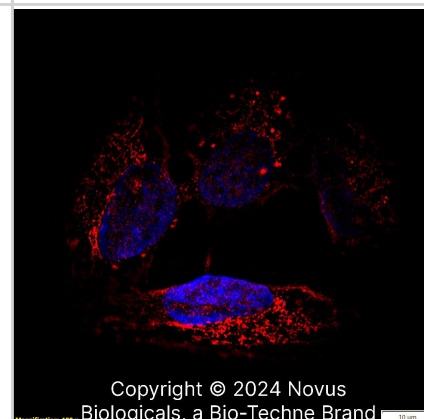
An intracellular stain was performed on A431 human skin carcinoma cell line using Mouse anti- CD63 (H5C6) Protein-G purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP2-42225AF647, blue histogram) or matched control antibody (orange histogram) at 2.5  $\mu\text{g}/\text{mL}$  for 30 minutes at RT.



Western Blot: CD63 Antibody (H5C6) - BSA Free [NBP2-42225] - NCAM as a neuronal exosome marker in plasma. (A–C) Quantitative analysis of plasma EVs using flow cytometry. Dot plots of fluorescent intensity for plasma EVs stained with PE-CY7-labeled CD63 antibody. EVs were unstained (A) & stained with either isotype control (B) or CD63 antibody (C). (D) A18945 iPSC-derived neurons cultured for 6 weeks & immunostaining with mature neuronal markers (DAPI, MAP2, NeuN, & merged image). Images were taken using Zeiss LSM confocal microscope at  $\times 63$  magnification. Scale bar, 20  $\mu\text{m}$ . (E) Representative images of cortical organoids at day 90 of differentiation. Scale bar, 1 mm. (F) The organoids express cortical layer marker BRN2 (also called POU3F2). The nuclei were stained with DAPI. Scale bar, 100  $\mu\text{m}$ . (Gi) Markers for proliferating neural progenitors (SOX2) & cortical neuron marker CTIP2 (also known as BCL11B) with nuclei DAPI staining (Gii). (H) Western blot analysis of NCAM & CD63 from EVs released from iPSCs, iPSC-derived cortical neurons, & iPSC-derived brain organoids. EVs were isolated using SEC from the cell culture media of each sample, & equal EV particle numbers ( $6 \times 10^8$ ) were subject to immunoblotting with NCAM & CD63 antibodies. (I–L) Flow cytometry dot plots of fluorescent intensity for plasma EVs double-stained with Dil & NCAM antibody. EV samples were unstained (I), single stained with Dil (J), & double-stained with Dil & NCAM antibody in the absence (K) & presence (L) of 0.1% Triton X-100. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/35655952>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



CD63 (H5C6) was detected in immersion fixed U-2 OS human osteosarcoma cell line using Mouse anti- CD63 (H5C6) Protein-G purified Monoclonal Antibody conjugated to Biotin (Catalog # NBP2-42225B) at 5  $\mu\text{g}/\text{mL}$  overnight at 4C. Cells were stained using Streptavidin conjugated to DyLight 550 (red) and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



## Publications

Proestler E, Donzelli J, Nevermann S et al. The multiple functions of miR-574-5p in the neuroblastoma tumor microenvironment *Frontiers in pharmacology* 2023-09-04 [PMID: 37731742] (Flow Cytometry, Immunoprecipitation, Western Blot, Human)

Rackles E, Zaccheroni E, Lopez PH, Faletti S et Al. Increased levels of circulating cell-free double-stranded nucleic acids in the plasma of glioblastoma patients *J Extracell Biol* 2024-08-05 [PMID: 39100684]

Genard GC, Tirinato L, Pagliari F et Al. Lipid droplets and small extracellular vesicles: More than two independent entities *J Extracell Biol* 2024-09-01 [PMID: 39257626]

Zhai C, Long J, He J et al. Precise Identification and Profiling of Surface Proteins of Ultra Rare Tumor Specific Extracellular Vesicle with Dynamic Quantitative Plasmonic Imaging *ACS nano* 2023-08-28 [PMID: 37638659]

Lucía Barrado-Gil, Isabel García-Dorival, Inmaculada Galindo, Covadonga Alonso, Miguel Ángel Cuesta-Geijo Insights into the function of ESCRT complex and LBPA in ASFV infection *Frontiers in Cellular and Infection Microbiology* 2023-12-06 [PMID: 38125905]

Ju Hyun Bae, Chan Hyeong Lee, Dokyung Jung, Kyungmoo Yea, Byoung Joon Song, Hakho Lee, Moon Chang Baek Extracellular vesicle isolation and counting system (EVics) based on simultaneous tandem tangential flow filtration and large field of view light scattering *Journal of Extracellular Vesicles* 2024-07-08 [PMID: 38978321]

Yiwei Ai, Chenxu Guo, Marta Garcia-Contreras, Laura S. Sánchez B., Andras Saftics, Oluwapelumi Shodubi, Shankar Raghunandan, Junhao Xu, Shang Jui Tsai, Yi Dong, Rong Li, Tijana Jovanovic-Talisman, Stephen J. Gould Endocytosis blocks the vesicular secretion of exosome marker proteins *Science Advances* 2024-05-10 [PMID: 38718108]

Matsui T, Sakamaki Y, Hiragi S, Fukuda M VAMP5 and distinct sets of cognate Q-SNAREs mediate exosome release *Cell structure and function* 2023-09-14 [PMID: 37704453]

Wang J, Trau M, Wuethrich A A Microfluidic SERS Assay to Characterize the Phenotypic Heterogeneity in Cancer-Derived Small Extracellular Vesicles *Methods in molecular biology (Clifton, N.J.)* 2023-06-10 [PMID: 37300621]

Saftics A, Abuelreich S, Romano E et al. Single Extracellular Vesicle Nanoscopy *Journal of extracellular vesicles* 2023-07-01 [PMID: 37422692] (DB, Human)

Details:  
1:500 dilution

Del Rivero T, Milberg J, Bennett C et al. Human amniotic fluid derived extracellular vesicles attenuate T cell immune response *Frontiers in immunology* 2022-11-28 [PMID: 36518766] (ICC/IF, Human)

de la Cruz-Ojeda P, Schmid T, Boix L et al. miR-200c-3p, miR-222-5p, and miR-512-3p Constitute a Biomarker Signature of Sorafenib Effectiveness in Advanced Hepatocellular Carcinoma Cells 2022-08-28 [PMID: 36078082] (WB, Human)

More publications at <http://www.novusbio.com/NBP2-42225>



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Free Phone: 0800 37 34 15  
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info.EMEA@bio-techne.com

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General: novus@novusbio.com

### **Products Related to NBP2-42225**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

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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 100% guarantee, please visit [www.novusbio.com/guarantee](http://www.novusbio.com/guarantee)

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