

# 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)



[technical@novusbio.com](mailto:technical@novusbio.com)

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Updated 12/20/2023 v.20.1

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**NBP2-42225**

CD63 Antibody (H5C6) - BSA Free

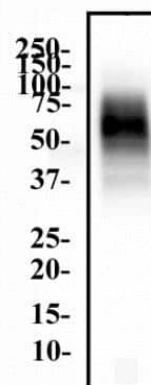
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	H5C6
Preservative	0.05% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	967
Gene Symbol	CD63
Species	Human, Canine
Marker	Exosome Marker
Immunogen	Human splenic adherent cells.

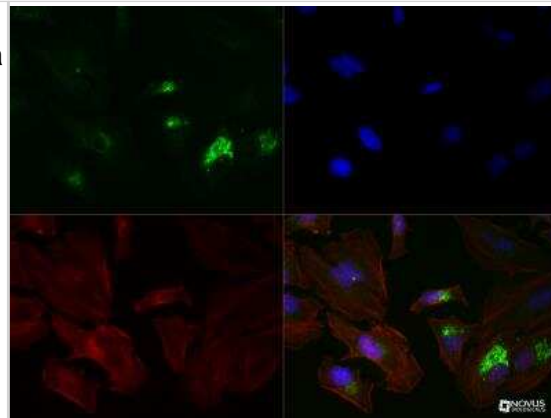
Product Application Details	
Applications	Western Blot, Dot Blot, ELISA, Electron Microscopy, Flow Cytometry, Flow (Intracellular), Functional, Immunocytochemistry/ Immunofluorescence, In vitro assay, Immunoprecipitation, Block/Neutralize, Immunohistochemistry Whole-Mount
Recommended Dilutions	Western 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**

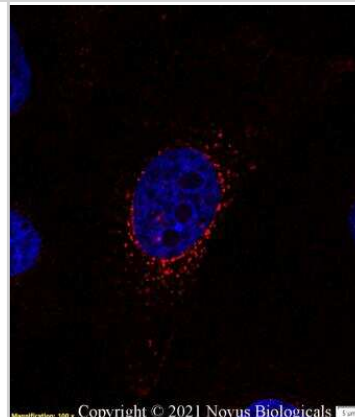
Western Blot: CD63 Antibody (H5C6) [NBP2-42225] - THP1 whole cell protein was separated by SDS-PAGE on a 12% gel and transferred to PVDF membrane. The membrane was probed with anti-CD63 antibody at 2 ug/mL and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



**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.

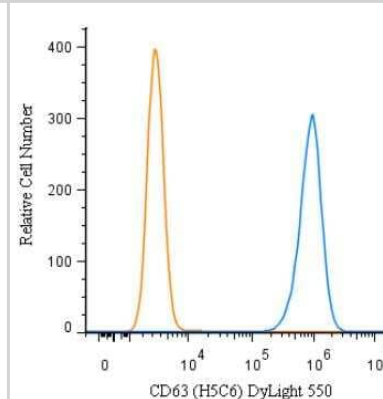


**Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225]** - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-CD63 Antibody [H5C6] conjugated to DyLight 550 (NBP2-42225R) at 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.



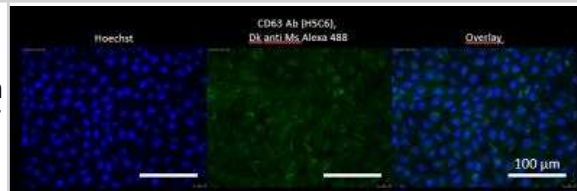
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**Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225]** - An intracellular stain was performed on SK-MEL-28 cells with CD63 (H5C6) Antibody NBP2-42225R (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized 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.

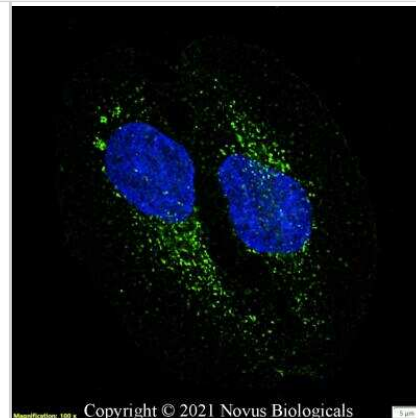


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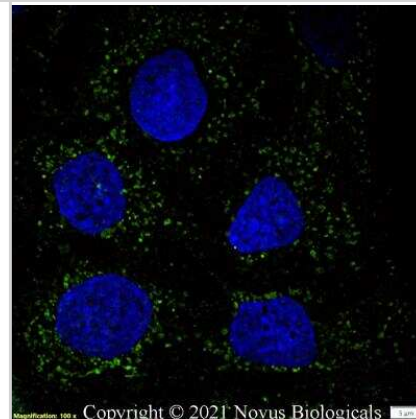
**Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225]** - MDCK cells stained with CD63 antibody at a dilution of 1:50 followed by Donkey anti-mouse secondary antibody conjugated with Alexa Fluor 488 (1:500). Nuclei were stained with Hoechst 33342. ICC/IF image submitted by a verified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



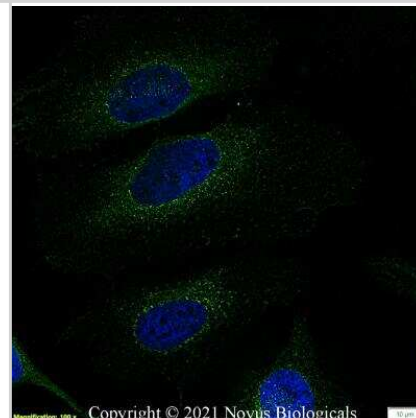
Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - U2OS cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-CD63 Antibody [H5C6] conjugated to Alexa Fluor 488 (NBP2-42225AF488) 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.



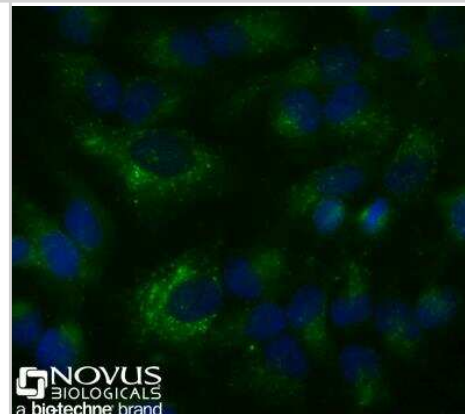
Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-CD63 Antibody [H5C6] conjugated to Biotin (NBP2-42225B) at 5 ug/ml for 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.



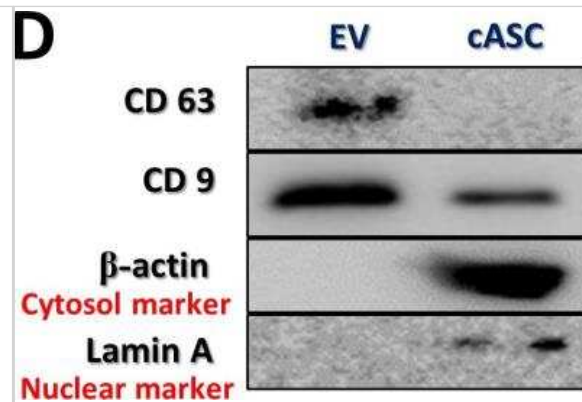
Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-CD63 Antibody [H5C6] conjugated to Biotin (NBP2-42225B) at 5 ug/ml for 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.



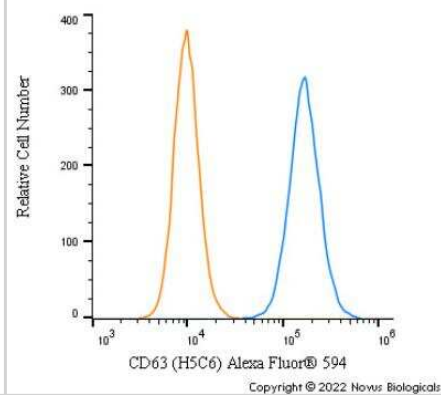
Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-CD63 [H5C6] conjugated to Alexa Fluor 488 [NBP2-42225AF488] at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



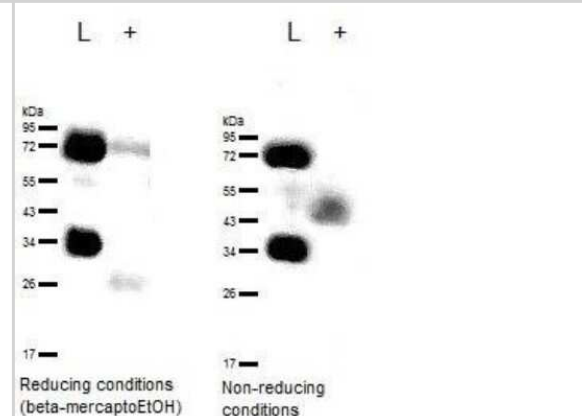
**Western Blot: CD63 Antibody (H5C6) - BSA Free [NBP2-42225]** - Analysis of common EV markers, where 10 ug of total protein was loaded in each lane. cASCs-EVs expressed CD63 and CD9, while beta actin and lamin A showed lower expression. The displayed data represent at least three repeated experiments 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.



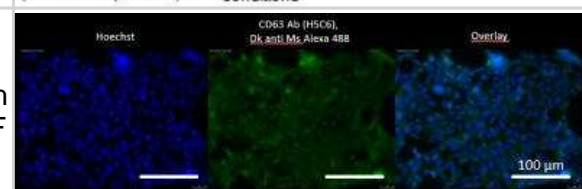
**Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225]** - An intracellular stain was performed on HeLa cells with CD63 [H5C6] Antibody NBP2-42225AF594 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody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



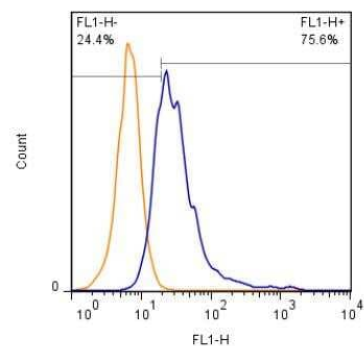
**Western Blot: CD63 Antibody (H5C6) [NBP2-42225]** - The same samples and volumes were run under reducing and non-reducing conditions. All procedures were performed in parallel for both conditions. L- ladder +- exosomes. Image visualized with HRP linked to secondary antibody. Western blot image submitted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



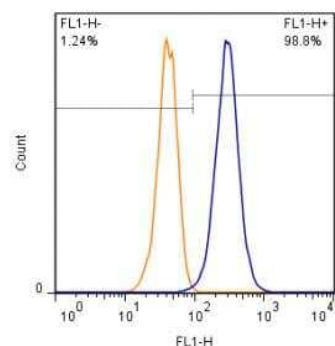
**Immunocytochemistry/Immunofluorescence: CD63 Antibody (H5C6) [NBP2-42225]** - HEK cells stained with CD63 antibody at a dilution of 1:50 followed by Donkey anti-mouse secondary antibody conjugated with Alexa Fluor 488 (1:500). Nuclei were stained with Hoechst 33342. ICC/IF image submitted by a verified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



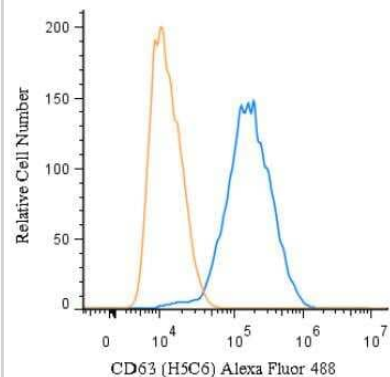
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - Human peripheral blood cells were stained ( $2 \times 10^6$  cells/mL) using the anti-CD63 antibody (Blue) at a dilution of 1:1000. Signal was detected using a Gt x Ms DyLight 488 Secondary and gated to the monocyte/granulocyte cell populations. Isotype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



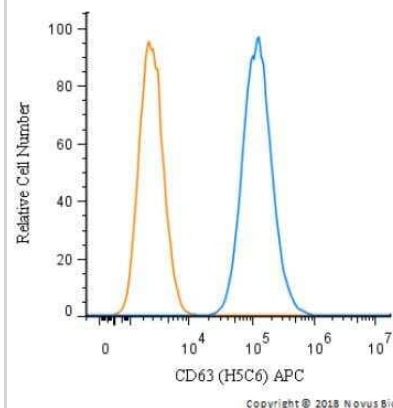
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - A431 cells were stained ( $1 \times 10^6$  cells/mL) using the anti-CD63 antibody at a 1:1000 dilution (blue). Signal was detected with Gt x Ms DyLight 488 secondary. Isotype control (orange).



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  $\mu$ g/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.

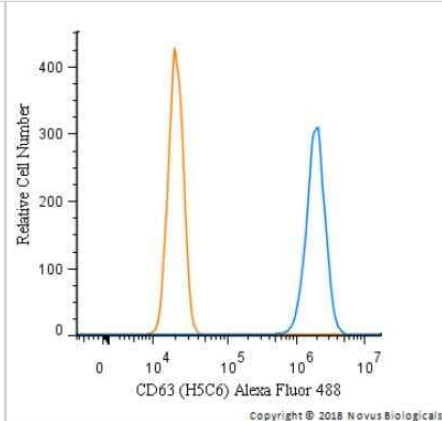


Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - An intracellular stain was performed on HeLa cells with CD63 Antibody (H5C6) NBP2-42225APC (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution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

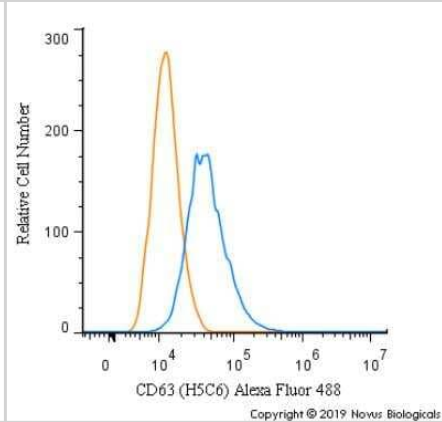


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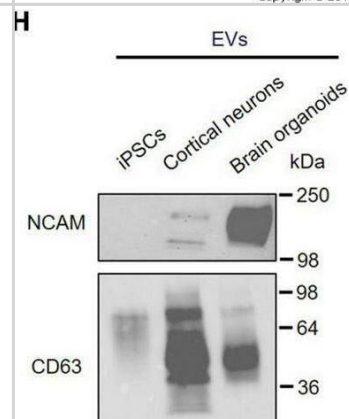
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - An intracellular stain was performed on SK-MEL-28 cells with CD63 Antibody (H5C6) NBP2-42225AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody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



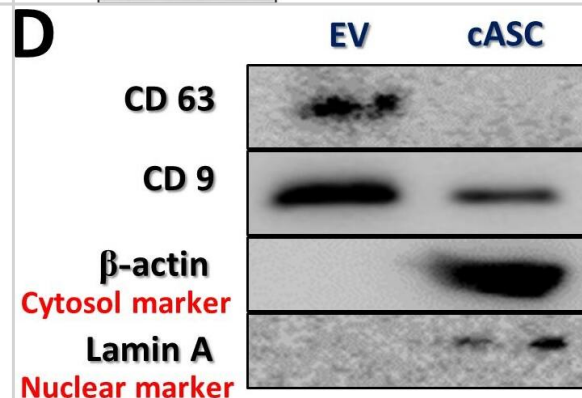
Flow Cytometry: CD63 Antibody (H5C6) [NBP2-42225] - An intracellular stain was performed on HeLa cells with CD63 [H5C6] Antibody NBP2-42225AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution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



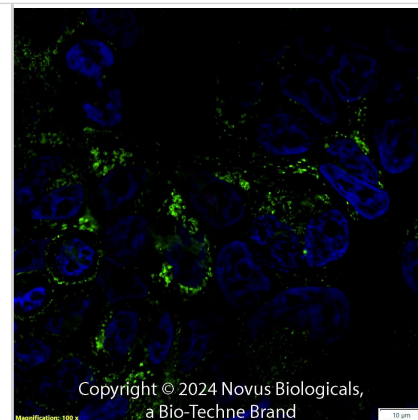
NCAM as a neuronal exosome marker in plasma. (H) Western blot analysis of NCAM and CD63 from EVs released from iPSCs, iPSC-derived cortical neurons, and iPSC-derived brain organoids. EVs were isolated using SEC from the cell culture media of each sample, and equal EV particle numbers ( $6 \times 10^8$ ) were subject to immunoblotting with NCAM and CD63 antibodies. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/35655952>), licensed under a CC-BY licence.



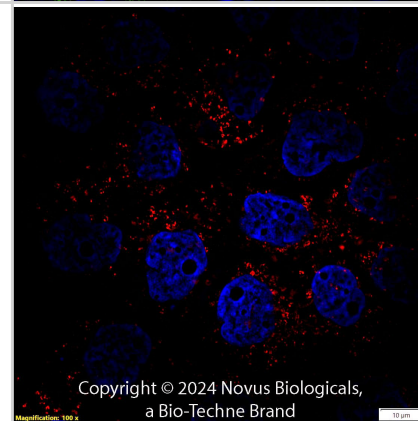
Characterization of cASC-EVs. (D) Immunoblotting analysis of common EV markers, where 10  $\mu\text{g}$  of total protein was loaded in each lane. cASCs-EVs expressed CD63 and CD9, while beta actin and lamin A showed lower expression. The displayed data represent at least three repeated experiments with consistent results. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32040478>), licensed under a CC-BY licence.



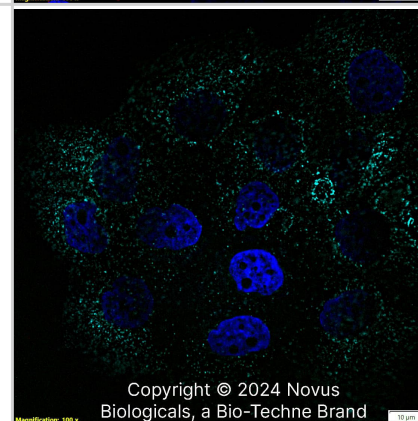
CD63 (H5C6) was detected in immersion fixed MCF7 human breast cancer cell line using Mouse anti-CD63 (H5C6) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NBP2-42225AF488) (green) at 5 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged 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 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 Alexa Fluor® 647 (Catalog # NBP2-42225AF647) (light blue) at 2 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.





## Publications

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]

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] (WB, Human)

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)

Ali Moussa HY, Manaph N, Ali G et al. Single Extracellular Vesicle Analysis Using Flow Cytometry for Neurological  
Disorder Biomarkers *Frontiers in integrative neuroscience* 2022-05-17 [PMID: 35655952] (WB, Human)

Details:

Dilutions: 1:1000

Bernuz M, Pallares-Rusinol A, Rossi R et al. Magnetic Separation of Cell-Secreted Vesicles with Tailored Magnetic  
Particles and Downstream Applications *Methods in molecular biology (Clifton, N.J.)* 2023-05-04 [PMID: 37140802]

Lin W, Yuan L, Gao Z et al. An Integrated Sample-to-Answer SERS Platform for Multiplex Phenotyping of  
Extracellular Vesicles *SSRN Electronic Journal* 2023-01-28

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





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Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

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

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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)
NBP2-42225AF488	CD63 Antibody (H5C6) [Alexa Fluor® 488]

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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.

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

