

Product Datasheet

rRNA Antibody (Y10b) - BSA Free NB100-662

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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NB100-662

rRNA Antibody (Y10b) - BSA Free

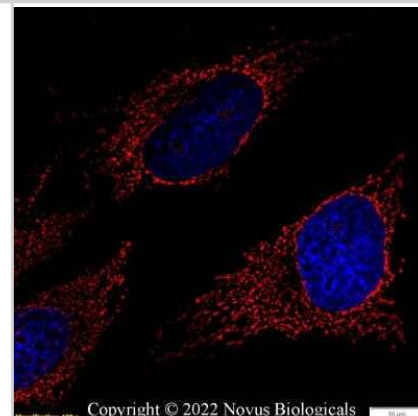
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Monoclonal
Clone	Y10b
Preservative	0.02% Sodium Azide
Isotype	IgG3 Kappa
Purity	Protein A purified
Buffer	PBS

Product Description	
Host	Mouse
Species	Human, Mouse, All Species, Bacteria
Reactivity Notes	This antibody reacts with all eukaryotes. Bacteria reactivity reported in scientific literature (PMID: 26134566).
Specificity/Sensitivity	This is specific for ribosomal RNA. There is no cross-reactivity with other RNAs.
Immunogen	The whole 5.8S ribosomal RNA.

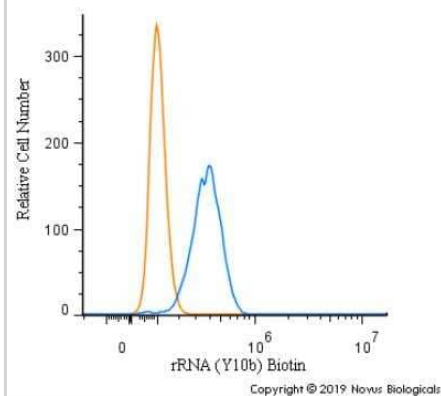
Product Application Details	
Applications	Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, CyTOF-ready, CyTOF-reported
Recommended Dilutions	Flow Cytometry 2.5 ug/ml, Immunohistochemistry 1:100 - 1:300, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 34980897), Immunoprecipitation 10 ul/lysate, Immunohistochemistry-Paraffin 1:100 - 1:300, Flow (Intracellular) 2.5 ug/ml, CyTOF-reported, CyTOF-ready reported by customer review
Application Notes	In ICC/IF, staining was observed in Ribosomes in the nucleoli and cytoplasm of Ntera2 cells.

Images

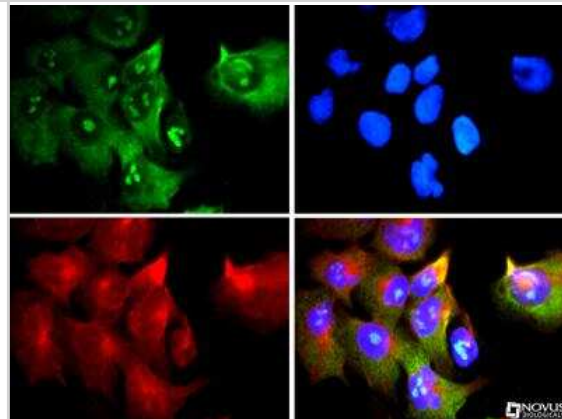
Immunocytochemistry/Immunofluorescence: rRNA Antibody (Y10b) [NB100-662] - HeLa cells were fixed and permeabilized for 10 minutes with -20C MeOH. The cells were incubated with rRNA Antibody [Y10b] conjugated to Biotin (NB100-662B) at 5ug/ml overnight at 4C and detected with Streptavidin conjugated to DyLight 550. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



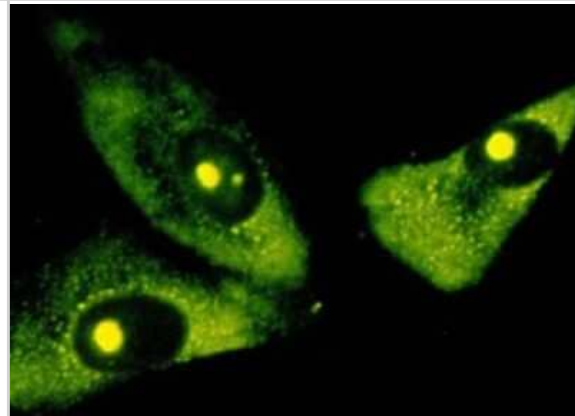
Flow Cytometry: rRNA Antibody (Y10b) [NB100-662] - An intracellular stain was performed on HeLa cells with rRNA Antibody [Y10b] NB100-662B (blue) and a matched isotype control (orange). Both antibodies were conjugated to Biotin. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature, followed by Streptavidin - R-Phycoerythrin Protein (2012-1000, Novus Biologicals).



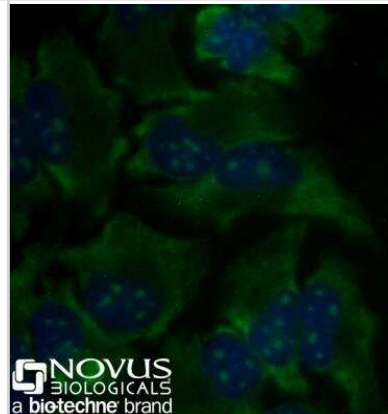
Immunocytochemistry/Immunofluorescence: rRNA Antibody (Y10b) [NB100-662] - rRNA antibody was tested in Ntera-2 cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



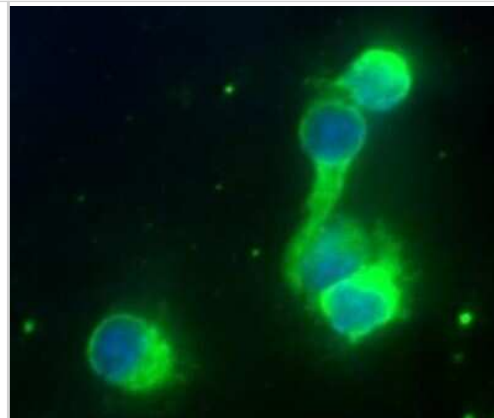
Immunocytochemistry/Immunofluorescence: rRNA Antibody (Y10b) [NB100-662] - Staining of rRNA in vero cells.



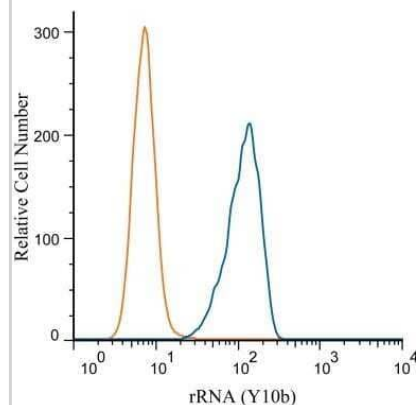
Immunocytochemistry/Immunofluorescence: rRNA Antibody (Y10b) [NB100-662] - HeLa cells were fixed and permeabilized for 10 minutes using -20C MeOH. The cells were incubated with anti-rRNA (Y10b) conjugated to DyLight 488 [NB100-662G] at 5ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



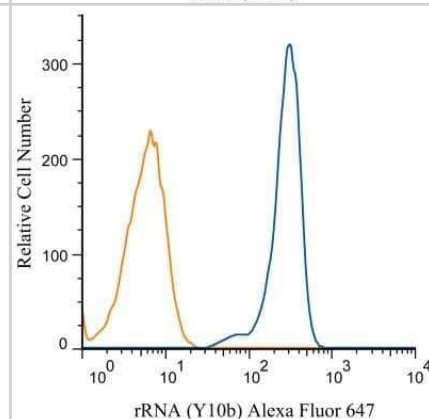
Immunocytochemistry: rRNA Antibody (Y10b) [NB100-662] - Immunohistochemical staining of mouse neuronal progenitor culture using rRNA antibody (green). Image from verified customer review.



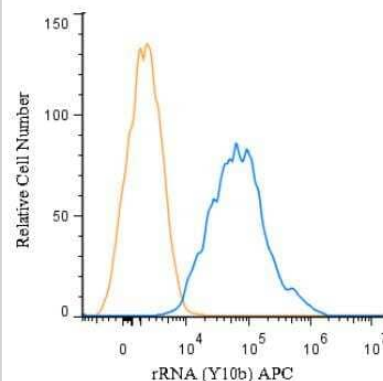
Flow (Intracellular): rRNA Antibody (Y10b) [NB100-662] - Analysis using Alexa Fluor (R) 488 conjugate of NB100-662. An intracellular stain was performed on HeLa cells with rRNA antibody (Y10b) NB100-662 (blue) and an isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% 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 488.



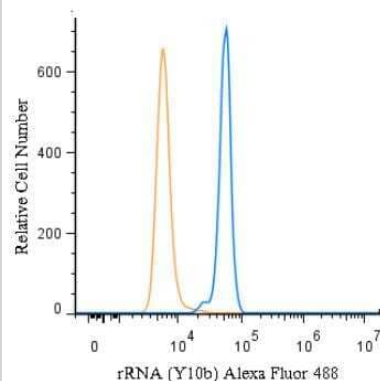
Flow (Intracellular): rRNA Antibody (Y10b) [NB100-662] - Analysis of Alexa Fluor (R) 647 conjugate of NB100-662. An intracellular stain was performed on HeLa cells with rRNA (Y10b) antibody NB100-662AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1%



Flow (Intracellular): rRNA Antibody (Y10b) [NB100-662] - An intracellular stain was performed on RAW 246.7 cells with rRNA Antibody (Y10b) NB100-662APC (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.

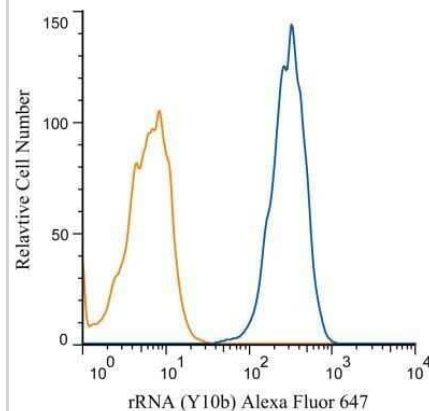


Flow (Intracellular): rRNA Antibody (Y10b) [NB100-662] - An intracellular stain was performed on A549 cells with NB100-662AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.

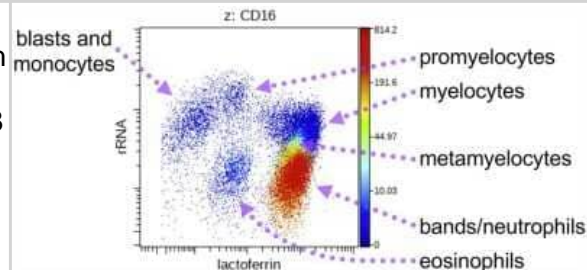


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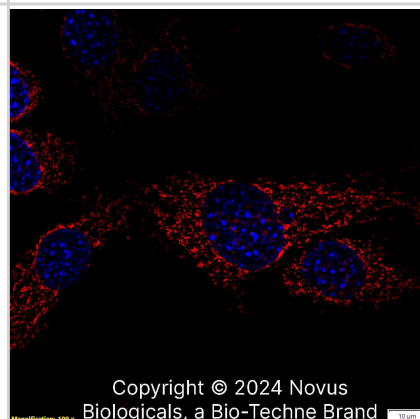
Flow (Intracellular): rRNA Antibody (Y10b) [NB100-662] - An intracellular stain was performed on SH-SY5Y cells with rRNA (Y10b) antibody NB100-662AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



CytoTOF-reported: rRNA Antibody (Y10b) [NB100-662] - Human bone marrow after RBC lysis, stained with anti-CD16, fixed, permeabilized with methanol, stained with anti-lactoferrin 1C6 NB120-10109 and anti-rRNA Y10b NB100-662, and gated on myeloid cells per Nat Med. 2020. 26:408-417. CyTOF image submitted by a verified customer review.

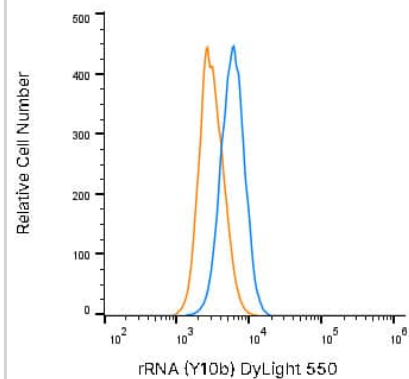


rRNA (Yb10) was detected in immersion fixed NIH3T3 Mouse fibroblast cell line using Mouse anti- rRNA (Yb10) Protein G Purified Monoclonal Antibody conjugated to Biotin (Catalog # NB100-662B) at 5 µg/mL overnight at 4C. Cells were stained using Streptavidin conjugated to DyLight 550 (red) and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.

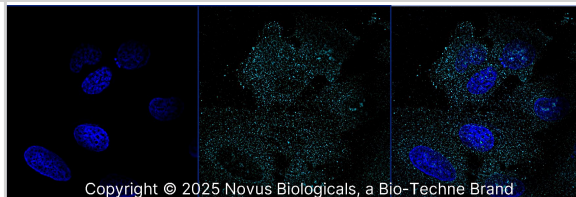


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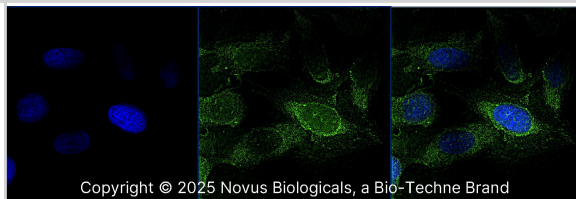
An intracellular stain was performed on A431 human skin carcinoma cell line using Mouse anti-rRNA (Y10b) Protein-G purified Monoclonal Antibody conjugated to DyLight 550 (Catalog # NB100-662R, blue histogram) or matched control antibody (orange histogram) at 2.5 µg/mL for 30 minutes at RT.



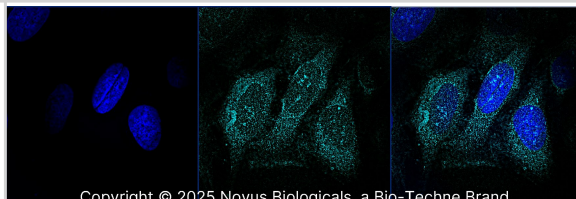
rRNA (Yb10) was detected in immersion fixed U-2 OS human osteosarcoma cell line using Mouse anti- rRNA (Yb10) Protein G Purified Monoclonal Antibody conjugated to DyLight 650 (Catalog # NB100-662C) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



rRNA (Yb10) was detected in immersion fixed U-2 OS human osteosarcoma cell line using Mouse anti-rRNA (Yb10) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NB100-662AF488) (green) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



rRNA (Yb10) was detected in immersion fixed U-2 OS human osteosarcoma cell line using Mouse anti-rRNA (Yb10) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB100-662AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

Caterino C, Ugolini M, Durso W et al. Translational Remodeling of the Synaptic Proteome During Aging. *Aging cell* 2025-10-16 [PMID: 41099377]

Zhang H, Chen Q, Zhang Q et al. DDX24 Mutation Alters NPM1 Phase Behavior and Disrupts Nucleolar Homeostasis in Vascular Malformations *International Journal of Biological Sciences* 2023-08-06 [PMID: 37705750] (Immunoprecipitation, Mouse)

Hodgson RE, Rayment JA, Huang WP, Sanchez Avila A et Al. C9orf72 poly-PR forms anisotropic condensates causative of nuclear TDP-43 pathology *iScience* 2024-10-11 [PMID: 39391721]

Ortega, JA;Sasselli, IR;Bocchitto, M;Fleming, AC;Fortuna, TR;Li, Y;Sato, K;Clemons, TD;Mckenna, ED;Nguyen, TP;Anderson, EN;Asin, J;Ichida, JK;Pandey, UB;Wolin, SL;Stupp, SI;Kiskinis, E; CLIP-Seq analysis enables the design of protective ribosomal RNA bait oligonucleotides against C9ORF72 ALS/FTD poly-GR pathophysiology *Science advances* 2023-11-10 [PMID: 37948524]

Lee KH, Zhang P, Kim HJ, Mitrea DM et Al. C9orf72 Dipeptide Repeats Impair the Assembly, Dynamics, and Function of Membrane-Less Organelles *Cell* 2016-10-22 [PMID: 27768896]

Chen L, Gai X, Yu X. et Al. Pre-rRNA facilitates the recruitment of RAD51AP1 to DNA double-strand breaks *J Biol Chem* 2024-02-24 [PMID: 38403248]

Søren Lykke-Andersen, Britt Kidmose Ardal, Anne Kruse Hollensen, Christian Kroun Damgaard, Torben Heick Jensen Box C/D snoRNP Autoregulation by a cis-Acting snoRNA in the NOP56 Pre-mRNA. *Molecular cell* 2019-04-15 [PMID: 30220559]

Pietraforte I, Butera A, Gaddini L et al. CXCL4-RNA Complexes Circulate in Systemic Sclerosis and Amplify Inflammatory/Pro-Fibrotic Responses by Myeloid Dendritic Cells *International Journal of Molecular Sciences* 2022-12-30 [PMID: 36614095] (WB, Human)

Vivanco Gonzalez N, Oliveria JP, Tebaykin D et al. An optimized protocol for phenotyping human granulocytes by mass cytometry STAR protocols [PMID: 35434655]

Gai X, Xin D, Wu D et al. Pre-ribosomal RNA reorganizes DNA damage repair factors in nucleus during meiotic prophase and DNA damage response *Cell research* 2022-01-04 [PMID: 34980897] (ICC/IF)

Fischer S, Nasyrov E, Brosien M Et al. Self-extracellular RNA promotes pro-inflammatory response of astrocytes to exogenous and endogenous danger signals *Journal of neuroinflammation* 2021-11-02 [PMID: 34727934] (IF/IHC)

Smolka JA, Sanz LA, Hartono SR, ChEdin F Recognition of RNA by the S9.6 antibody creates pervasive artifacts when imaging RNA:DNA hybrids *The Journal of cell biology* 2021-06-07 [PMID: 33830170]

More publications at <http://www.novusbio.com/NB100-662>

Procedures

Immunocytochemistry Protocol Specific for rRNA Antibody (Y10b) (NB100-662)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.



Flow (Intracellular) Protocol for rRNA Antibody (NB100-662)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 μ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
 - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1×10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 μ L samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
 - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
 - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 μ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 μ g per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer + 0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 μ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 μ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 μ L per sample) and proceed with analysis on your flow cytometer.





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Products Related to NB100-662

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-96978	Mouse IgG3 Kappa Light Chain Isotype Control (MG3K)
NB100-662AF488	rRNA Antibody (Y10b) [Alexa Fluor® 488]

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

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