Product Datasheet

Nox4 Antibody - BSA Free
NB110-58851

Unit Size: 0.1 ml

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

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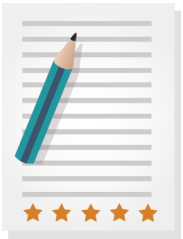
Updated 10/23/2024 v.20.1



NB110-58851

Nox4 Antibody - 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	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

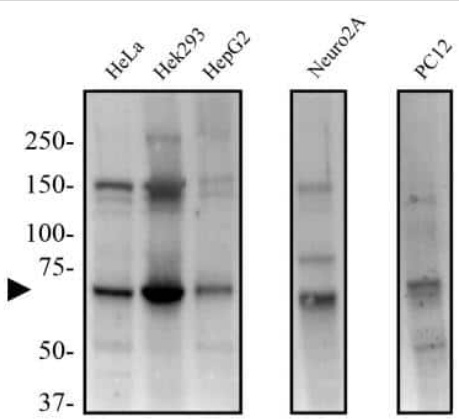
Host	Rabbit
Gene ID	50507
Gene Symbol	NOX4
Species	Human, Mouse, Rat, Porcine, Primate
Immunogen	A synthetic peptide made to a C-terminal region (within residues 500-578) of the human NOX4 protein sequence. [Swiss-Prot# Q9NPH5].

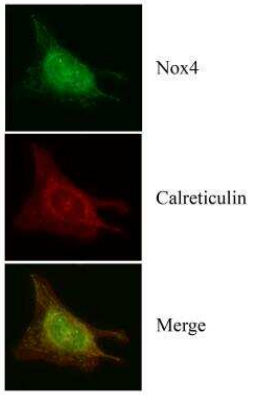
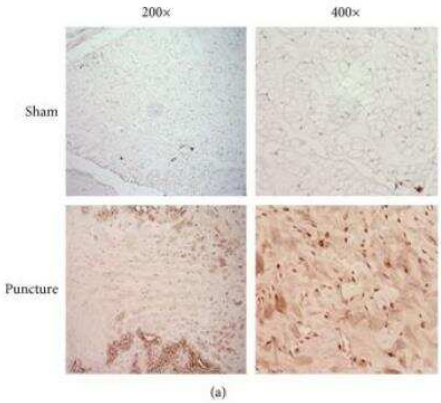
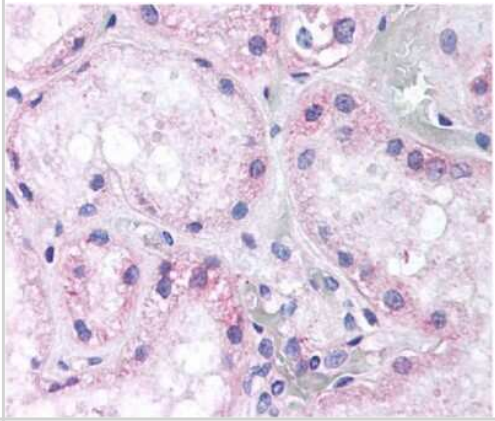
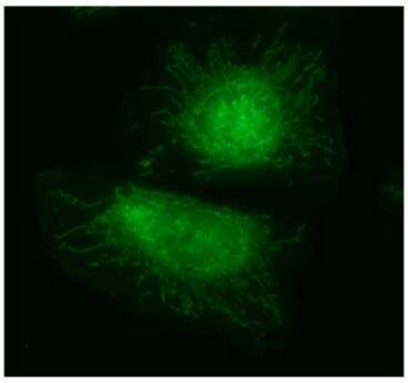
Product Application Details

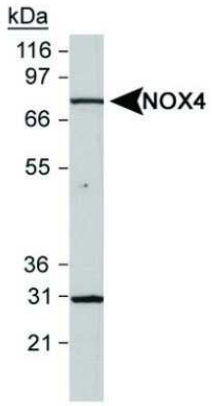
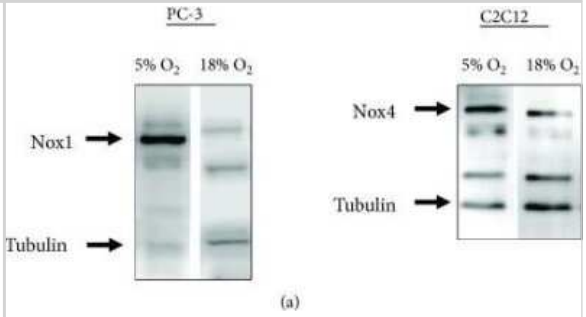
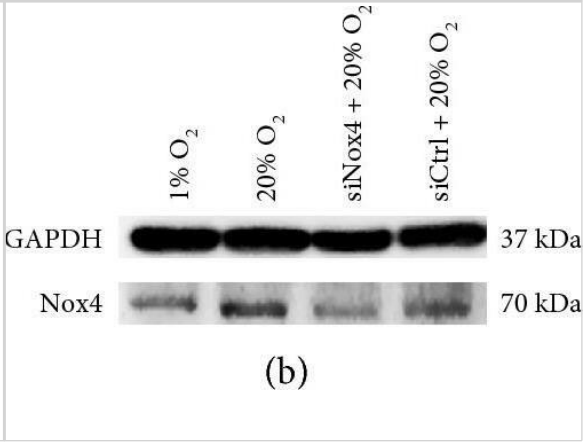
Applications	Western Blot, Simple Western, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 2 - 4 ug/ml, Simple Western, Flow Cytometry, Immunohistochemistry 10 - 20 ug/ml, Immunocytochemistry/ Immunofluorescence 10 - 20 ug/ml, Immunoprecipitation reported in scientific literature (PMID 25062272), Immunohistochemistry-Paraffin 10 - 20 ug/ml, Flow (Intracellular)
Application Notes	In Western Blot this NOX4 antibody recognizes bands at ~70kDa. See Simple Western Antibody Database for Simple Western validation: Tested in pancreas; separated by size; antibody dilution of 1:1000.

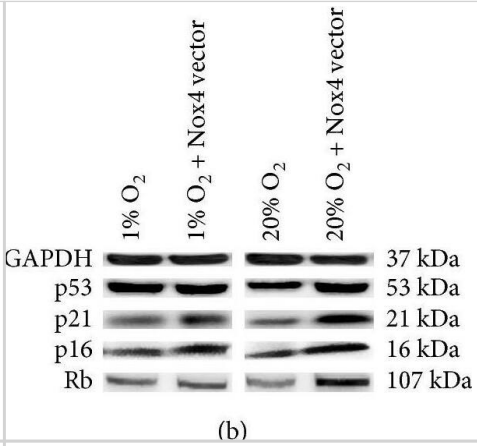
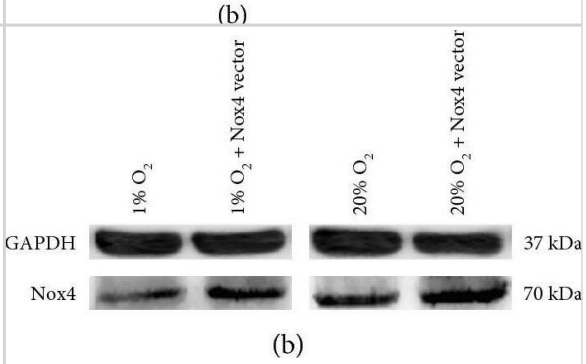
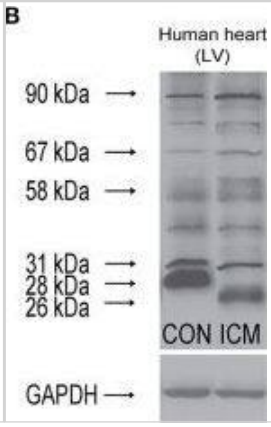
Images

Western Blot: Nox4 Antibody [NB110-58851] - Whole cell protein from human HeLa, Hek293, HepG2, mouse Neuro2A and rat PC12 cells was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% BSA in TBST. The membrane was probed with 2.0 ug/ml anti-Nox4 in 1% BSA and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Nox4 is detected at approx. 70 kDa (arrowhead)



Immunocytochemistry/Immunofluorescence: Nox4 Antibody [NB110-58851] - HeLa cells were fixed in 10% buffered formalin for 10 min and permeabilized in 0.1% Triton X-100 in PBS for 10 min. Cells were incubated with antibodies to Nox4 (NB110-58851) and the ER marker Calreticulin (NBP1-47518), each at 20 ug/ml for 1 hour at room temperature. The coverslips were washed 3x in PBS and incubated with Alexa-Fluor 488 anti-rabbit secondary antibody. (green) and DyLight 550 anti-mouse secondary antibody. The merged image shows the co-localization of Nox4 and Calreticulin in the ER.	
Immunohistochemistry: Nox4 Antibody [NB110-58851] - The expression of Nox4, p21, and Rb was upregulated in degenerative discs. Immunohistochemical staining for Nox4 in NP specimens. Image collected and cropped by CiteAb from the following publication (https://www.hindawi.com/journals/omcl/2017/7426458/) licensed under a CC-BY license.	
Immunohistochemistry: Nox4 Antibody [NB110-58851] - Analysis using the Biotin conjugate of NB110-58851. Staining of NOX4 in proximal convoluted tubules of human kidney.	
Immunocytochemistry/Immunofluorescence: Nox4 Antibody [NB110-58851] - HeLa cells were fixed in 10% buffered formalin for 10 min and permeabilized in 0.1% triton X-100 in PBS for 10 min. Cells were incubated with NB110-58851 at 20 ug/ml for 1 hour at room temperature, washed 3x in PBS and incubated with Alexa-Fluor 488 anti-rabbit secondary antibody. Nox4 (Green) was detected in the ER.	

Western Blot: Nox4 Antibody [NB110-58851] - Alternative splicing of NOX4 in rat kidney and heart. Alternative splicing of NOX4 in human hearts. Image collected and cropped by CiteAb from the following publication (https://journal.frontiersin.org/article/10.3389/fphys.2017.00935/full), licensed under a CC-BY license.	
Western Blot: Nox4 Antibody [NB110-58851] - Analysis using the Biotin conjugate of NB110-58851. Detection of NOX4 in human kidney lysates uisng NB110-58851 at 0.5 ug/ml. Band observed at 31 kDa may represent reported splice isoform.	
Western Blot: Nox4 Antibody - BSA Free [NB110-58851] - Nox4 Antibody [NB110-58851] - Reduced levels of NADPH oxidases 1 and 4 at 18% versus 5% O2. Representative Western blots showing Nox1 and beta-tubulin in PC-3 cells or Nox4 and beta-tubulin in C2C12 cells, at 5% and 18% O2. Image collected and cropped by CiteAb from the following publication (https://www.hindawi.com/journals/omcl/2018/8238459/), licensed under a CC-BY license.	
Western Blot: Nox4 Antibody - BSA Free [NB110-58851] - Small interfering RNA against Nox4 (siNox4) alleviated oxidative stress & DNA damage induced by high oxygen tension in NP cells. (a, b) RT-qPCR analysis (N = 3) & representative immunoblot analysis of Nox4 in NP cells. The knockdown of Nox4 in NP cells was confirmed. (c) ROS production in NP cells (N = 3). (d) RT-qPCR analysis of MsrbA, MsrB1, & MsrB2 in NP cells (N = 3). (e, f) Immunofluorescence staining of γ-H2A.X & percentage of γ-H2A.X-positive cells in NP cells (N = 6). NP cells were transfected with siNox4 or scrambled siRNA control (siCtrl) before high oxygen tension treatment. □, P value < 0.05, error bars represent standard error. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29147462), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: Nox4 Antibody - BSA Free [NB110-58851] - Nox4 overexpression accelerated senescence of NP cells. (a, b) RT-qPCR analysis (N = 4) & representative immunoblot analysis of p53, p16, p21, & Rb in NP cells overexpressing Nox4. (c) The percentage of SA-β-gal-positive NP cells overexpressing Nox4 (N = 8). (d, e) Immunofluorescence staining of BrdU & percentage of BrdU-positive cells in NP cells overexpressing Nox4 (N = 8). (f, g) RT-qPCR analysis of matrix degradation enzymes & proinflammatory cytokines in NP cells overexpressing Nox4 (N = 4). NP cells were transfected with Nox4 vectors for Nox4 overexpression. □, P value < 0.05, error bars represent standard error. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29147462), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: Nox4 Antibody - BSA Free [NB110-58851] - Nox4 overexpression boosted ROS production & induced DNA damage in NP cells. (a) RT-qPCR analysis (N = 4) of Nox4, MsrB1, & MsrB2 in NP cells overexpressing Nox4. (b) Representative immunoblot analysis of Nox4 in NP cells overexpressing Nox4. (c) ROS production in NP cells overexpressing Nox4 (N = 3). (d, e) Immunofluorescence staining of γ-H2A.X & percentage of γ-H2A.X-positive cells in NP cells overexpressing Nox4 (N = 8). NP cells were transfected with Nox4 vectors for Nox4 overexpression. □, P value < 0.05, error bars represent standard error. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29147462), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: Nox4 Antibody - BSA Free [NB110-58851] - Alternative splicing of NOX4 in rat kidney & heart (A). Alternative splicing of NOX4 in human hearts (B). Quantitative evaluation of spliced NOX4 isoforms in ICM samples (C). Quantitative evaluation of spliced NOX4 isoforms in DCM samples (D). Data are mean ± S.E.M. n = 5/group. *p < 0.05. LV, left ventricle; IVS, interventricular septum; RV, right ventricle; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29204124), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Liheng Kang, Meihua Piao, Nan Liu, Wanping Gu, Chunsheng Feng Sevoflurane Exposure Induces Neuronal Cell Ferroptosis Initiated by Increase of Intracellular Hydrogen Peroxide in the Developing Brain via ER Stress ATF3 Activation Molecular Neurobiology 2023-10-24 [PMID: 37874483]

Wang N, Peng Y, Su X et al. Histone Deacetylase 5 Is an Early Epigenetic Regulator of Intermittent Hypoxia Induced Sympathetic Nerve Activation and Blood Pressure Frontiers in Physiology 2021-05-17 [PMID: 34079475]

Hwang S, Kim SH, Yoo KH et al. Exogenous 8-hydroxydeoxyguanosine attenuates doxorubicin-induced cardiotoxicity by decreasing pyroptosis in H9c2 cardiomyocytes BMC molecular and cell biology 2022-12-14 [PMID: 36517746]

Diebold BA, Wilder SG, De Deken X et al. Guidelines for the Detection of NADPH Oxidases by Immunoblot and RT-qPCR Methods Mol. Biol. 2019-06-08 [PMID: 31172474]

Wing-Kee Lee, Stephanie Probst, Bettina Scharner, Timo Deba, Faouzi Dahdouh, Frank Thévenod Distinct concentration-dependent oxidative stress profiles by cadmium in a rat kidney proximal tubule cell line Archives of Toxicology 2024-01-30 [PMID: 38289529]

Arias-Cavieres A, Garcia AJ A Consequence of Immature Breathing induces Persistent Changes in Hippocampal Synaptic Plasticity and Behavior: A Role of Pro-Oxidant State and NMDA Receptor Imbalance bioRxiv : the preprint server for biology 2023-03-21 [PMID: 36993632] (WB, Mouse)

Kang L, Piao M, Liu N et al. Sevoflurane exposure induces neuronal cell ferroptosis initiated by increase of intracellular hydrogen peroxide in the developing brain via ER stress ATF3 activation Research Square 2023-05-18 (Western Blot)

Mellone M, Piotrowska K, Venturi G et al. ATM Regulates Differentiation of Myofibroblastic Cancer-Associated Fibroblasts and Can Be Targeted to Overcome Immunotherapy Resistance Cancer research 2022-11-10 [PMID: 36353752] (ICC/IF, Human)

Tang P, Sheng J, Peng X et al. Targeting NOX4 disrupts the resistance of papillary thyroid carcinoma to chemotherapeutic drugs and lenvatinib Cell death discovery 2022-04-08 [PMID: 35396551] (WB, Human)

Ke G, Chen X, Liao R et al. Receptor Activator of NF- kappa B Mediates Podocyte Injury in Diabetic Nephropathy Kidney international 2021-05-26 [PMID: 34051263]

Vandenberg GG, Dawson NJ, Head A et al. Astrocyte-mediated disruption of ROS homeostasis in Fragile X mouse model Neurochemistry international 2021-03-27 [PMID: 33785420] (WB, Human)

Garcla-Arroyo FE, Tapia E, MuNoz-JimEnez I et al. Fluid Intake Restriction Concomitant to Sweetened Beverages Hydration Induce Kidney Damage Oxidative medicine and cellular longevity 2020-12-02 [PMID: 33354281] (WB, Rat)

More publications at http://www.novusbio.com/NB110-58851

Procedures

Western Blot Protocol for Nox4 Antibody (NB110-58851)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three 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 manufacturers instructions.

Immunocytochemistry/Immunofluorescence Protocol for Nox4 Antibody (NB110-58851)
Immunocytochemistry Protocol

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Immunohistochemistry-Paraffin Protocol for Nox4 Antibody (NB110-58851)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Flow (Intracellular) Protocol for Nox4 Antibody (NB110-58851)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 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 uL 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 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL 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 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 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 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug 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 uL 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 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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Products Related to NB110-58851

NB820-59231	Human Kidney Whole Tissue Lysate (Adult Whole Normal)
NB110-58851PEP	Nox4 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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