Product Datasheet

TRPA1 Antibody - BSA Free
NB110-40763

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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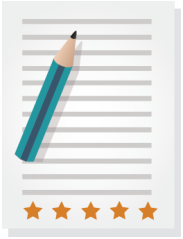
Updated 5/6/2024 v.20.1



NB110-40763

TRPA1 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	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	127.5 kDa

Product Description

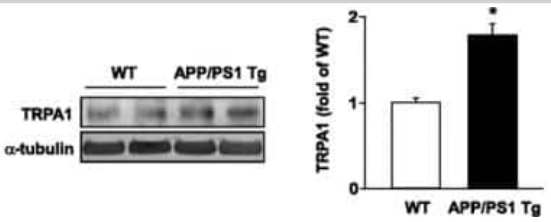
Host	Rabbit
Gene ID	8989
Gene Symbol	TRPA1
Species	Human, Mouse, Rat, Guinea Pig, Zebrafish
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 27748654). Zebrafish reactivity reported from a verified customer review.
Specificity/Sensitivity	Additional modified form of TRPA1 can also be detected.
Immunogen	A synthetic peptide made to a region within the N-terminus (residues 1-100) of the human TRPA1 protein. [Swiss-Prot# O75762]

Product Application Details

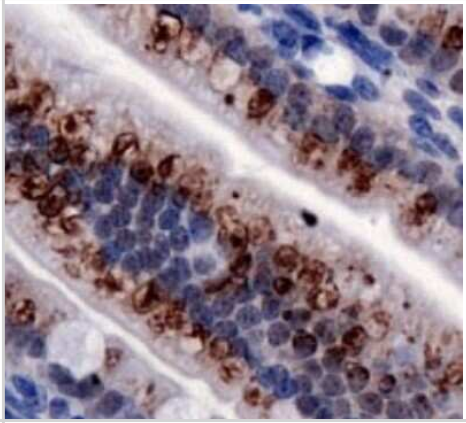
Applications	Western Blot, ELISA, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 2 ug/ml, Flow Cytometry reported in scientific literature (PMID 28990934), ELISA reported in scientific literature (PMID 29803505), Immunohistochemistry 0.5-1.0 ug/ml, Immunocytochemistry/ Immunofluorescence 1:200, Immunohistochemistry-Paraffin 1:100-1:250, Immunohistochemistry-Frozen reported in scientific literature, Flow (Intracellular)

Images

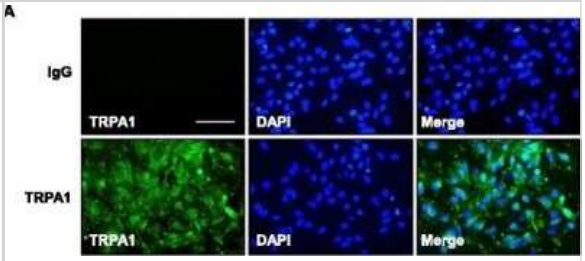
Western Blot: TRPA1 Antibody [NB110-40763] - Expression and localization of TRPA1 channels in wild-type (WT) and APP/PS1 Tg mice. Brains were harvested from WT and APP/PS1 Tg mice at 8A months old. Western blot analysis of protein levels of TRPA1 and I+--tubulin. Image collected and cropped by Citeab from the following publication (Role of transient receptor potential ankyrin 1 channels in Alzheimer's disease. J Neuroinflammation (2016)) licensed under a CC-BY license.



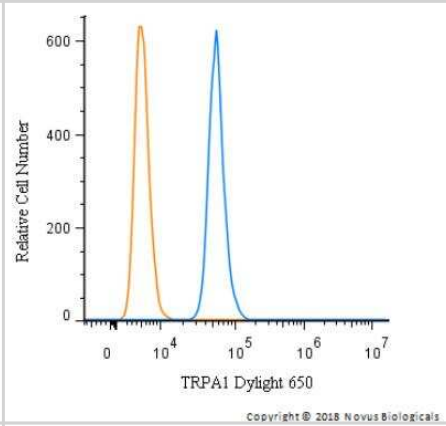
Immunohistochemistry: TRPA1 Antibody [NB110-40763] - Staining TRPA1 in mouse intestine.



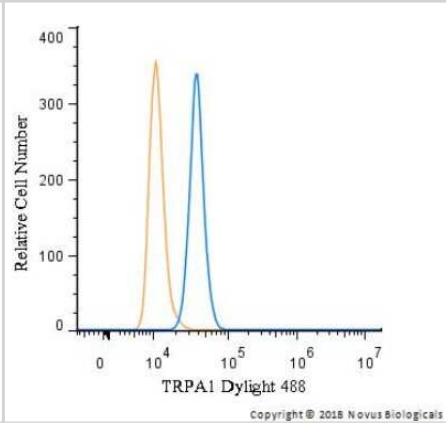
Immunocytochemistry/Immunofluorescence: TRPA1 Antibody [NB110-40763] - Ai2 elicits TRPA1-dependent Ca2+ influx in astrocytes. Immunostaining of primary astrocytes from WT mice with anti-IgG and anti-TRPA1 antibody, then FITC-conjugated secondary antibody. Image collected and cropped by Citeab from the following publication (Role of transient receptor potential ankyrin 1 channels in Alzheimer's disease. J Neuroinflammation (2016)) licensed under a CC-BY license.



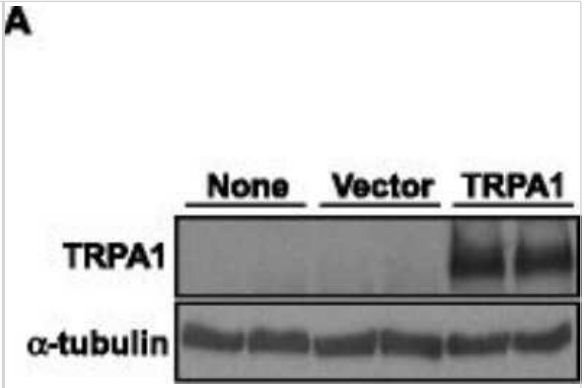
Flow Cytometry: TRPA1 Antibody [NB110-40763] - An intracellular stain was performed on A549 cells with NB110-40763C (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 650.



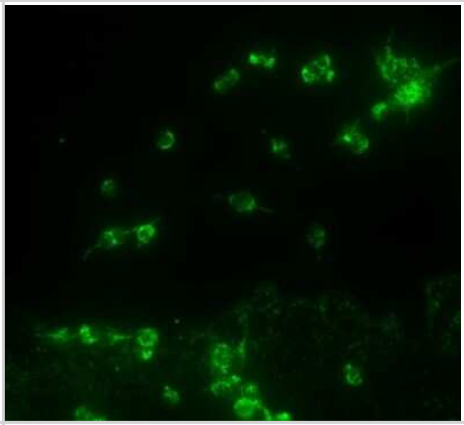
Flow Cytometry: TRPA1 Antibody [NB110-40763] - An intracellular stain was performed on A549 cells with NB110-40763G (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 488



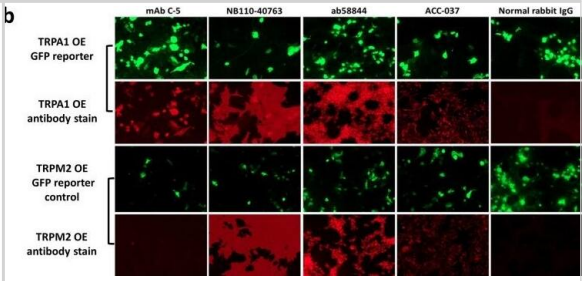
Western Blot: TRPA1 Antibody [NB110-40763] - The essential role of TRPA1 in Ai2-induced calcium influx in HEK293 cells. (a) Western blot analysis of TRPA1 and I+--tubulin protein levels in non-treated, vector-or TRPA1-transfected HEK293 cells. Image collected and cropped by Citeab from the following publication (Role of transient receptor potential ankyrin 1 channels in Alzheimer's disease. J Neuroinflammation (2016)) licensed under a CC-BY license.



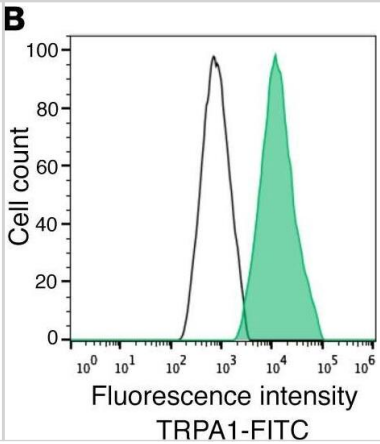
Immunocytochemistry/Immunofluorescence: TRPA1 Antibody [NB110-40763] - Analysis of TRPA1 in HEK 293 cells (Flp In Trex system) cells.

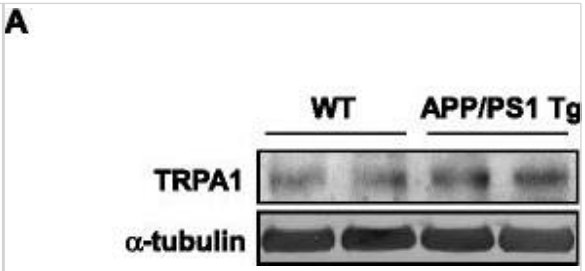
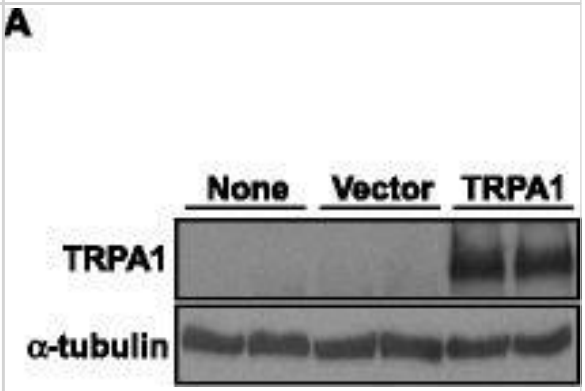


Evaluation of anti-TRPA1 antibody specificity by western blotting and immunofluorescence. b) The performance of the same antibodies was evaluated by immunofluorescence (red) in comparison to the expression of the GFP reporter (green), or control TRPM2 and GFP overexpressing cells as for western blotting. Mouse mAb C-5 again shows high specificity with a strong correlation between the expression of the GFP reporter and antibody staining, and no staining in TRPM2 and GFP overexpressing controls. NB110-40763 also demonstrates some sensitivity but has high background that likely does not correspond to Fc binding of the antibody, as the other two rabbit polyclonal antibodies or isotype control did show different staining patterns. Ab58844 and ACC-037 only appear to detect antigens other than TRPA1 in the conditions used. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31811235), licensed under a CC-BY licence.

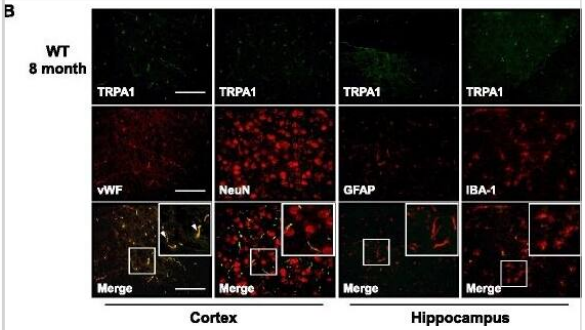


Oxygen-induced degranulation of mast cells was induced by TRPA1.(B and C) To identify O2-sensing molecules on mast cells, TRPA1 was detected by flow cytometry and immunoblotting. Results are representative of 3 independent experiments. A green peak shows a TRPA1-positive reaction and an open peak shows reactivity of a control (B). Instead of first Abs, rabbit serum was used as a control. Strong positive reactions that were visualized around 110 kDa (indicated by an arrow) were estimated as TRPA1 proteins (C). Lane 1, mouse brain membrane; lane 2, C57BL/6 BMCMCs. GAPDH was used as a control for one of the cytosolic endogenous proteins. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28990934), licensed under a CC-BY licence.



The production of DSBs depends on Top1 degradation in CPT-treated quiescent cells. (A–C) Serum-starved WI38 hTERT cells were co-transfected with siRNAs against cullin 3 and cullin 4B or against a control sequence and then treated with DMSO (−CPT) or 25 μM CPT (+CPT) for 1 h. (A and B) Western blotting of the indicated proteins. αTubulin: loading control. (C) Number of γH2AX foci per nucleus from one representative experiment (246–348 nuclei were analyzed for each treatment) out of three. ***P < 0.001. (D and E) Serum-starved WI38 hTERT cells were treated with DMSO or MG132 (50 μM) for 1 h before exposure to 0.8 Gy IR. One hour post-irradiation, cells were co-stained for γH2AX (green) and 53BP1 (red). (D) Representative pictures. (E) Number of γH2AX foci per nucleus from one representative experiment (162–180 nuclei were analyzed for each treatment) out of three. Ns: not significant. (F and G) U2OS EV28 cells were treated with DMSO or MG132 (10 μM) for 1 h before the addition of ethanol (untreated) or 300 nM 4-hydroxitamoxifen (4OHT) for 4 h to express AsiSI in the nucleus (42). (F) Representative pictures of cells co-stained for γH2AX (green) and 53BP1 (red). (G) ChIP analysis using an anti-γH2AX antibody (black) or a non-immune antibody (IgG, gray). Enrichment was assessed by QPCR amplification using primers proximal to two AsiSI sites located inside two genes (Gene I: SFRS6, Gene II: CCD47) and primers distal to an AsiSI site (Control). Enrichment was normalized to the maximum recovery for each experiment (means ± SEM, n = 3). Ns: not significant; *P < 0.05. In the microscopic images, nuclear contours, identified by DAPI staining (not shown), are indicated by dashed lines. Bars: 10 μm. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/26578593), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
dsRNA-induced SGs require G3BP1 and G3BP2, whereas RLBs do not.A, immunoblot for G3BP1, RNase L, GAPDH in parental (WT), RL-KO, G3BP-KO, and RL/G3BP-KO U-2 OS cells. B, immunoblot for G3BP1 and G3BP2 in RL-KO and RL/G3BP-KO U-2 OS cells. C, IF for G3BP1 and PABPC1 4 h post-poly(I·C) transfection or treatment with 500 μm sodium arsenite (SA) in the indicated cell lines. D, similar to C but enlarged to show RLB formation in U-2 OS-G3BP1/2-KO cells. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31896577), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

SOD2 mRNA and protein expression. RT-PCR: (A) SOD2 mRNA expression is higher in adults, and highest in the adult parenchyma. (B) No exposure effects on SOD2 mRNA were observed in neonates. (C) Adult SOD2 mRNA was decreased in PFP48. Data are presented as mean+SEM (n=5-7 rats/group, in each compartment), * significantly different compared to neonates in the same compartment, † significantly different compared to airways in the same age, ‡ significantly different compared to FA in the same compartment. Western blotting: (D) Scan of representative SOD2 and actin blots. (E) Neonatal whole lung SOD2 protein expression was unchanged with exposure, and (F) adult whole lung SOD2 protein trended upwards at PFP2, but was statistically insignificant. (G-J) Immunohistochemical localization of SOD2 in lung (n=6 rats/group). SOD2 protein was more abundant in adults compared to neonates, but no exposure specific differences were observed. Scale bar is 50 μm. Image collected and cropped by CiteAb from the following open publication (https://particleandfibretoxicology.biomedcentral.com/articles/10.1186/1743-8977-10-34), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Masaki Matsubara, Yukiko Muraki, Hiroka Suzuki, Noriyuki Hatano, Katsuhiko Muraki Critical amino acid residues regulating TRPA1 Zn 2+ response: A comparative study across species The Journal of Biological Chemistry 2024-04-18 [PMID: 38642892]

Kwang-Hyun Hur, Youyoung Lee, Audrey Lynn Donio, Seon-Kyung Kim, Bo-Ram Lee, Jee-Yeon Seo, Dooti Kundu, Kyeong-Man Kim, Stephen J Kohut, Seok-Yong Lee, Choon-Gon Jang Transient receptor potential ankyrin 1 channel modulates the abuse-related mechanisms of methamphetamine through interaction with dopamine transporter. British journal of pharmacology 2024-04-21 [PMID: 38644533]

Chihiro Soma, Suzuro Hitomi, Eri Oshima, Yoshinori Hayashi, Kumi Soma, Ikuko Shibuta, Yoshiyuki Tsuboi, Tetsuo Shirakawa, Takashi Kikuiri, Koichi Iwata, Masamichi Shinoda Involvement of oxidative stress in orofacial mechanical pain hypersensitivity following neonatal maternal separation in rats Scientific Reports 2023-12-20 [PMID: 38123836]

Clara Hoebart, Natalia S Rojas-Galvan, Cosmin I Ciotu, Ibrahim Aykac, Lukas F Reissig, Wolfgang J Weninger, Attila Kiss, Bruno K Podesser, Michael J M Fischer, Stefan Heber No functional TRPA1 in cardiomyocytes. Acta physiologica (Oxford, England) 2021-08-23 [PMID: 33819369]

Warfield R, Robinson JA, Podgorski RM et al. Neuroinflammation in the Dorsal Root Ganglia and Dorsal Horn Contributes to Persistence of Nociceptor Sensitization in SIV-Infected Antiretroviral Therapy-Treated Macaques The American journal of pathology 2023-09-19 [PMID: 37734588] (IHC)

Chen LH, Yeh YM, Chen YF et al. Targeting interleukin-20 alleviates paclitaxel-induced peripheral neuropathy Pain 2020-06-01 [PMID: 32068666] (In Vivo)

Prandini P, De Logu F, Fusi C et al. Transient Receptor Potential Ankyrin 1 Channels Modulate Inflammatory Response in Respiratory Cells from Patients with Cystic Fibrosis American Journal of Respiratory Cell and Molecular Biology 2016-11-01 [PMID: 27281024] (ICC/IF)

Guan M, Ying S, Wang Y. Increased expression of transient receptor potential channels and neurogenic factors associates with cough severity in a guinea pig model BMC Pulmonary Medicine 2021-12-01 [PMID: 34078339]

Ma S, Wang DH. Knockout of Trpa1 accelerates age-related cardiac fibrosis and dysfunction PLOS ONE 2022-09-14 [PMID: 36103570] (WB)

Yoshida M, Yamamiya R, Shimizu Y, Yoshimura K. Transgenic Chlamydomonas Expressing Human Transient Receptor Potential Ankyrin 1 (TRPA1) Channels to Assess the Effect of Agonists and Antagonists Frontiers in Pharmacology 2020-09-29 [PMID: 33117171]

Luostarinen S, H□m□l□inen M, Moilanen E. Transient Receptor Potential Ankyrin 1 (TRPA1)-An Inflammation-Induced Factor in Human HaCaT Keratinocytes International Journal of Molecular Sciences 2021-03-24 [PMID: 33805042] (WB, IP)

S□nchez JC, Mu□oz LV, Galindo-M□rquez ML et al. Paclitaxel Regulates TRPA1 Function and Expression Through PKA and PKC Neurochemical Research 2023-01-01 [PMID: 36098890] (WB)

More publications at http://www.novusbio.com/NB110-40763

Procedures

Western Blot protocol for TRPA1 Antibody (NB110-40763)
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 manufacturer's instructions.

Immunocytochemistry/ Immunofluorescence Protocol for TRPA1 Antibody (NB110-40763)
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 TRPA1 Antibody (NB110-40763)
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 TRPA1 Antibody (NB110-40763)

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 NB110-40763

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NB110-40763C	TRPA1 Antibody [DyLight 650]

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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