Product Datasheet

STING/TMEM173 Antibody - BSA Free
NBP2-24683

Unit Size: 0.1 mg

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

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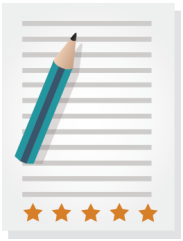
Updated 10/23/2024 v.20.1



NBP2-24683

STING/TMEM173 Antibody - BSA Free

Product Information

Unit Size	0.1 mg
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	Peptide affinity purified
Buffer	PBS
Target Molecular Weight	42 kDa

Product Description

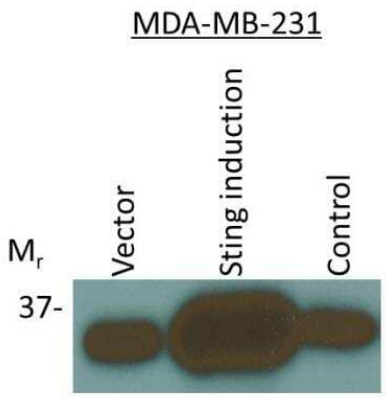
Host	Rabbit
Gene ID	340061
Gene Symbol	STING1
Species	Human, Mouse, Canine, Primate, Rhesus Macaque
Reactivity Notes	Opossum, Zebrafish (83%), Xenopus (72%), Rat (88%).
Immunogen	Partial synthetic peptide made to an internal portion of human STING/TMEM173 (between amino acids 310-360) [UniProt Q86WV6]

Product Application Details

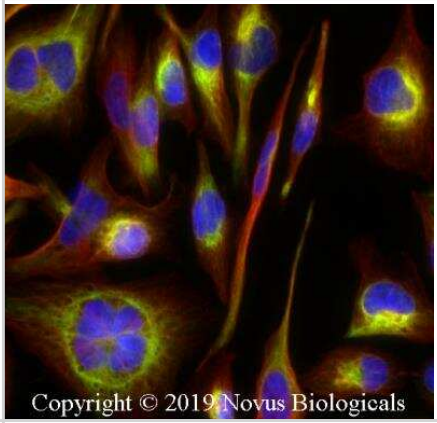
Applications	Western Blot, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockout Validated
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Flow Cytometry 1-5 ug/million cells, ELISA reported in scientific literature (PMID 34905508), Immunohistochemistry 1:100 - 1:300, Immunocytochemistry/ Immunofluorescence 5 - 10 ug/ml, Immunoprecipitation Validated for Immunoprecipitation from YCharOS Inc. (ycharos.com), Immunohistochemistry-Paraffin 1:100 - 1:300, Immunohistochemistry-Frozen reported in scientific literature (PMID 33745949), Knockout Validated Validated for Knockout from YCharOS Inc. (ycharos.com)

Images

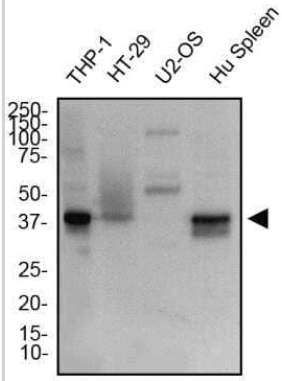
Western Blot: STING/TMEM173 Antibody [NBP2-24683] - STING/TMEM173 expression was induced in human breast MDA-MB-231 cells followed by Western blotting using STING/TMEM173 Antibody antibody (1:1000). Only one specific band at an apparent molecular mass of 37 kDa was observed. Image from verified customer review.



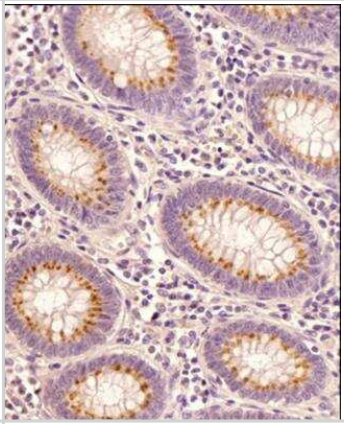
Immunocytochemistry/Immunofluorescence: STING/TMEM173 Antibody [NBP2-24683] - RH-30 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with STING/TMEM173 Antibody at 2 ug/mL overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha Tubulin Antibody (DM1A) (NB100-690) was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



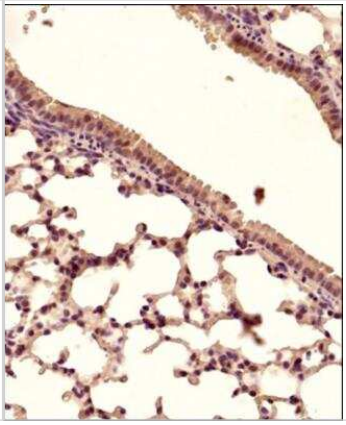
Western Blot: STING/TMEM173 Antibody [NBP2-24683] - Total protein from THP-1, HT-29, U2OS cells and human spleen was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml STING/TMEM173 Antibody in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.

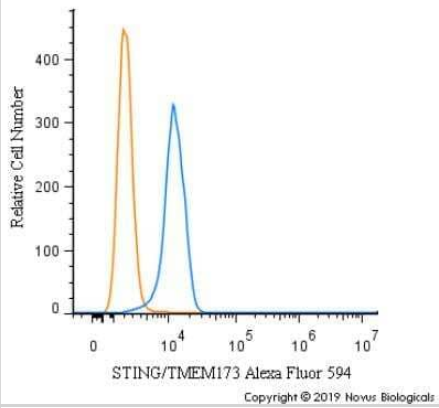
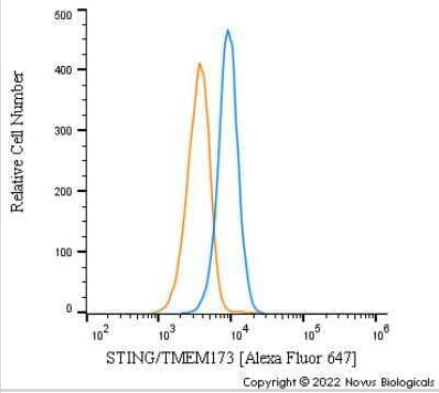
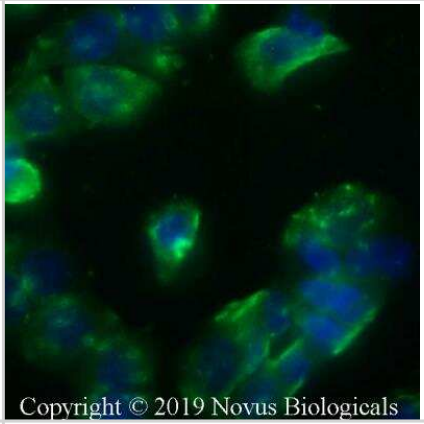
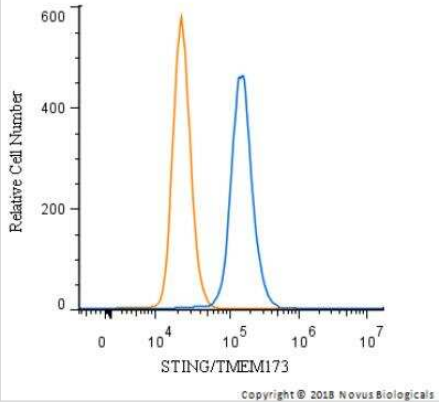


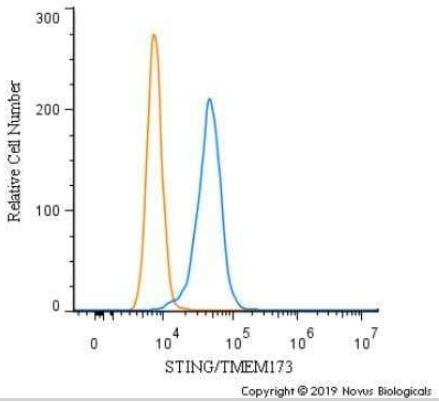
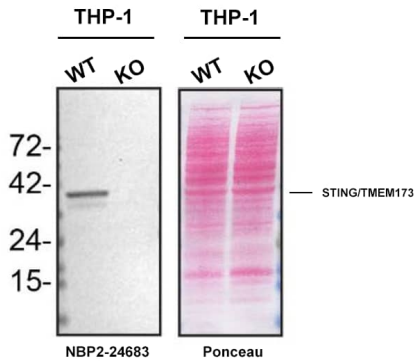
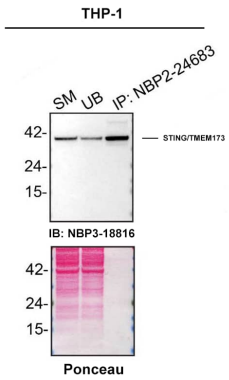
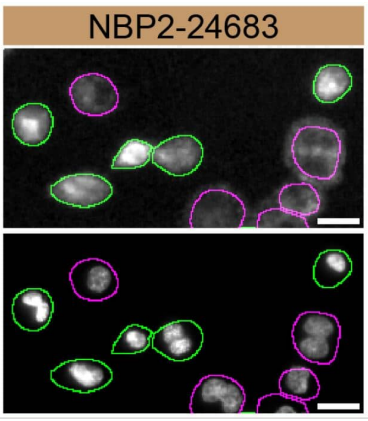
Immunohistochemistry-Paraffin: STING/TMEM173 Antibody [NBP2-24683] - Human colon cancer tissue section using STING/TMEM173 Antibody at 1:100 dilution with detection employing HRP-conjugated secondary antibody. The signal was developed using DAB reagent and the nuclei were counterstained with hematoxylin. The antibody generated very weak cytoplasmic staining in columnar epithelial cells with a very strong signal in the secretory/goblet cells.



Immunohistochemistry-Paraffin: STING/TMEM173 Antibody [NBP2-24683] - Mouse lung tissue section using STING/TMEM173 Antibody at 1:150 dilution with detection employing HRP-conjugated secondary antibody. The signal was developed using DAB reagent and the nuclei were counterstained with hematoxylin. The antibody generated mainly a cytoplasmic staining in the bronchiolar and alveolar epithelial cells.



Flow Cytometry: STING/TMEM173 Antibody [NBP2-24683] - An intracellular stain was performed on U937 cells with NBP2-24683AF594 (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.	
Flow Cytometry: STING/TMEM173 Antibody - BSA Free [NBP2-24683] - An intracellular stain was performed on THP-1 cells with STING/TMEM173 Antibody NBP2-24683AF647 (blue) and a matched isotype control NBP2-24891 (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.	
Immunocytochemistry/Immunofluorescence: STING/TMEM173 Antibody [NBP2-24683] - HT-29 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with STING/TMEM173 Antibody at 10 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Flow Cytometry: STING/TMEM173 Antibody [NBP2-24683] - An intracellular stain was performed on THP-1 cells with STING/TMEM173 Antibody and a matched isotype control. Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody.	

Flow Cytometry: STING/TMEM173 Antibody [NBP2-24683] - An intracellular stain was performed on RH30 cells with STING/TMEM173 Antibody (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, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody.	
Western blot shows lysates of THP-1 cell line and STING/TMEM173 knockout THP-1 cell line (KO). Nitrocellulose membrane was probed with STING/TMEM173 Antibody (Catalog # NBP2-24683) followed by an HRP-conjugated secondary antibody. A specific band was detected for STING/TMEM173 at approximately 41 kDa (as indicated) in the parental THP-1 cell line, but is not detectable in knockout THP-1 cell line. Primary antibody dilution used: 1/1000. The Ponceau stained transfer of the blot is shown. This experiment was conducted under reducing conditions. Image, protocol, and testing courtesy of YCharOS Inc. See ycharos.com for additional details.	
PMA-treated THP-1 lysates were prepared and immunoprecipitation was performed using 2.0 µg of STING/TMEM173 Antibody (Catalog # NBP2-24683) pre-coupled to Dynabeads protein A. Immunoprecipitated STING/TMEM173 was detected with STING/TMEM173 Antibody (Catalog # NBP3-18816). For western blot, NBP3-18816 was used at 1/1000. The Ponceau stained transfer of the blot is shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).	
PMA-treated THP-1 WT and STING/TMEM173 KO cells were labelled with a green or a far-red fluorescent dye, respectively. Cells were stained with STING/TMEM173 Antibody (Catalog # NBP2-24683) and with an Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the blue and red (grayscale) channels are shown. WT and KO cells are outlined with green and magenta dashed line, respectively. Primary antibody dilution used: 1/1000. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).	

Publications

Anh Cuong Hoang, László Sasi-Szabó, Tibor Pál, Tamás Szabó, Victoria Diedrich, Annika Herwig, Kathrin Landgraf, Antje Körner, Tamás Röszer Mitochondrial RNA stimulates beige adipocyte development in young mice Nature Metabolism 2022-11-28 [PMID: 36443525]

Pham AT, Oliveira AC, Albanna M et al. Non-Interferon-Dependent Role of STING Signaling in Pulmonary Hypertension Arteriosclerosis, thrombosis, and vascular biology 2023-11-09 [PMID: 37942608] (FLOW, Mouse, Human)

Varga K, Gyurina K, Radványi Á et al. Stimulator of Interferon Genes (STING) Triggers Adipocyte Autophagy Cells 2023-09-24 [PMID: 37830559] (IHC-P, Human)

Pantelidou C, Jadhav H, Kothari A et al. STING agonism enhances anti-tumor immune responses and therapeutic efficacy of PARP inhibition in BRCA-associated breast cancer npj Breast Cancer 2022-09-06 [PMID: 36068244] (Western Blot, Block/Neutralize)

Gong LK, Yang X, Yang J et al. Low-dose ganciclovir ameliorates dextran sulfate sodium-induced ulcerative colitis through inhibiting macrophage STING activation in mice Frontiers in Pharmacology 2022-11-17 [PMID: 36467059]

Liu H, Ghosh S, Vaidya T et al. Activated cGAS/STING signaling elicits endothelial cell senescence in early diabetic retinopathy JCI insight 2023-06-22 [PMID: 37345657] (ELISA)

Jing X, Luo X, Fang C, Zhang B N-acetylserotonin inhibits oxidized mitochondrial DNA-induced neuroinflammation by activating the AMPK/PGC-1?/TFAM pathway in neonatal hypoxic-ischemic brain injury model International Immunopharmacology 2023-03-01 (WB, IHC-P, Rat)

Ding R, Li H, Liu Y et al. Activating cGAS-STING axis contributes to neuroinflammation in CVST mouse model and induces inflammasome activation and microglia pyroptosis Journal of neuroinflammation 2022-06-10 [PMID: 35689216] (ICC/IF, Mouse)

Gupta U, Ghosh S, Wallace CT et al. Increased LCN2 (lipocalin 2) in the RPE decreases autophagy and activates inflammasome-ferroptosis processes in a mouse model of dry AMD Autophagy [PMID: 35473441]

Li X, Zhu Y, Zhang X et al. An alternatively spliced STING isoform localizes in the cytoplasmic membrane and directly senses extracellular cGAMP The Journal of clinical investigation 2021-12-14 [PMID: 34905508] (ELISA, WB, FLOW, Mouse)

Qu, H, Li, L Et al. Epithelial Cells in Endometriosis and Adenomyosis Upregulate STING Expression. Reprod Sci 2020 -06-01 [PMID: 32046461] (WB, Human)

Hoang A, Yu H, Lin Y Et al. Immune privilege of adipocyte mitochondria protects from obesity Research Square 2021-11-04 (FLOW)

More publications at http://www.novusbio.com/NBP2-24683

Procedures

Western Blot Protocol for STING/TMEM173 Antibody (NBP2-24683)
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 STING/TMEM173 Antibody (NBP2-24683)
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 STING/TMEM173 Antibody (NBP2-24683)
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 all the time).

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 STING/TMEM173 Antibody (NBP2-24683)

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 permeablization 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 permeabization 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.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NBP2-24683

NBP2-24683PEP	STING/TMEM173 Antibody Blocking 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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