Product Datasheet

LAMP-2/CD107b Antibody (H4B4) - BSA Free
NBP2-22217

Unit Size: 0.1 ml

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

Reviews: 2 Publications: 31

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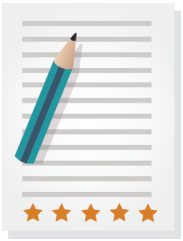
www.novusbio.com/NBP2-22217

Updated 4/13/2025 v.20.1



NBP2-22217

LAMP-2/CD107b Antibody (H4B4) - BSA Free

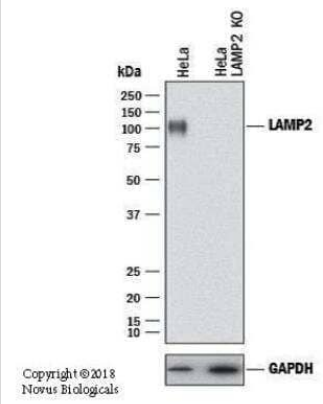
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	H4B4
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	45 kDa

Product Description	
Host	Mouse
Gene ID	3920
Gene Symbol	LAMP2
Species	Human, Mouse, Chicken, Rat (Negative)
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 27863209). Chicken reactivity reported in scientific literature (PMID: 30298003).
Marker	Late Endosome / Lysosome marker
Immunogen	LAMP-2/CD107b Antibody (H4B4) was made using a human adherent spleen cells.

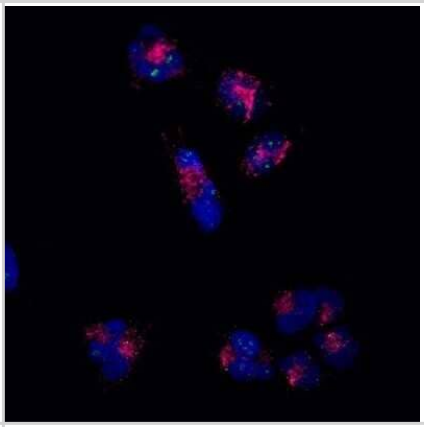
Product Application Details	
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, CyTOF-ready, Knockout Validated
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:25, Flow Cytometry 1 ug per million cells, ELISA, Immunohistochemistry 1:100 - 1:200, Immunocytochemistry/ Immunofluorescence 1:10-1:100, Immunohistochemistry-Paraffin 1:100 - 1:200, Immunohistochemistry-Frozen 1:100 - 1:200, Immunoblotting reported in scientific literature (PMID 29643474), CyTOF-ready, Knockout Validated
Application Notes	In Western blot, bands may be seen at ~40 kDa and 45 kDa representing the unglycosylated isoforms of LAMP2 and ~110 kDa representing the glycosylated form. In Simple Western only 10 - 15 ul of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 1.0 mg/mL, separated by Size, antibody dilution of 1:25, apparent MW was 41 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue. This antibody is CyTOF ready.

Images

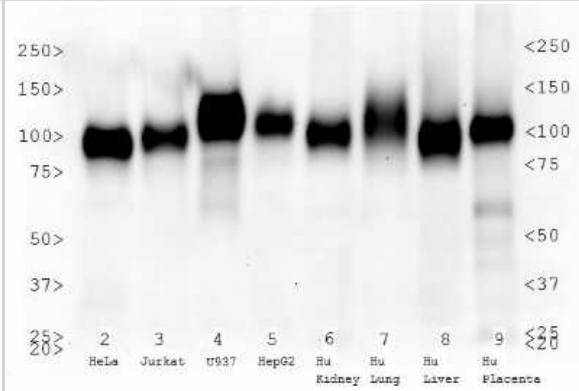
Western Blot: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - Lysates of HeLa human cervical epithelial carcinoma parental cell line and LAMP2 knockout (KO) HeLa cell line. PVDF membrane was probed with Mouse Anti-Human LAMP-2/CD107b (H4B4) Monoclonal Antibody (Catalog # NBP2-22217) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was detected for LAMP-2/CD107b at approximately 100 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. This experiment was conducted under reducing conditions.



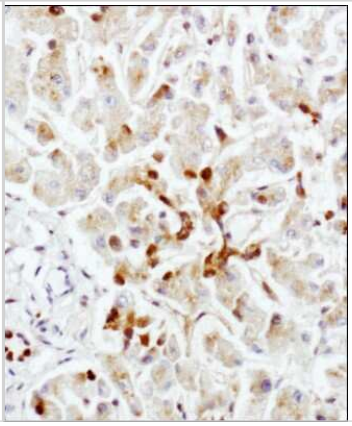
Immunocytochemistry/Immunofluorescence: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - Immunofluorescence: [Alexa Fluor® 647] [NBP2-22217AF647] - T98G glioblastoma cells grown on #1.5 coverglass stained with a conjugated LAMP-2/CD107b (H4B4)AF647 antibody, counterstained with DAPI. Cathepsin D (green) staining also visible. This image was submitted via customer Review. Image using the Alexa Fluor 647 form of this antibody.

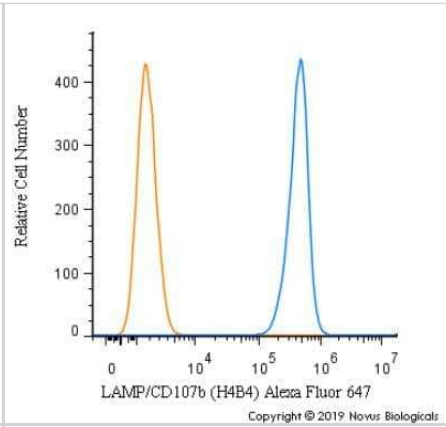
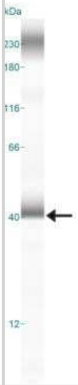
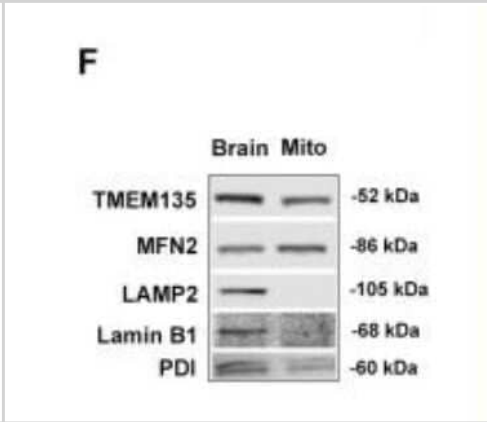
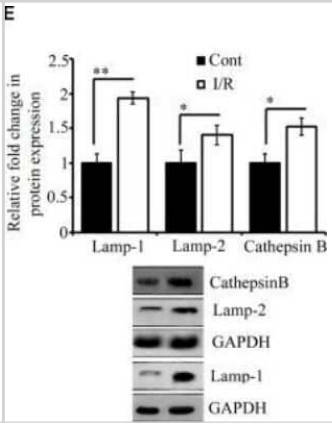


Western Blot: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - Analysis of LAMP-2/CD107b expression in Whole Cell Lysates: 2) HeLa, 3) Jurkat, 4) U937, 5) HepG2, Tissue Extracts: 6) Human kidney, 7) Human lung, 8) Human liver, and 9) Human placenta.

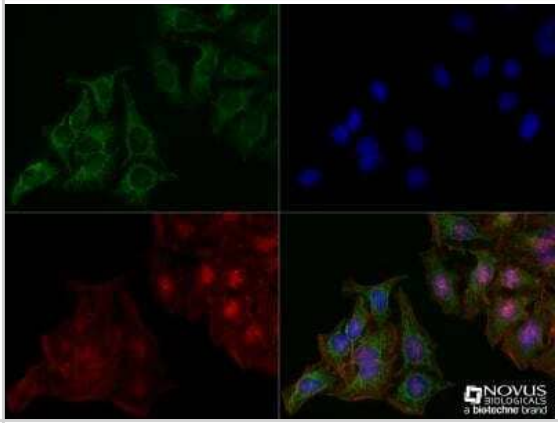


Immunohistochemistry-Paraffin: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - Staining of LAMP-2/CD107b in human liver using DAB with hematoxylin counterstain.

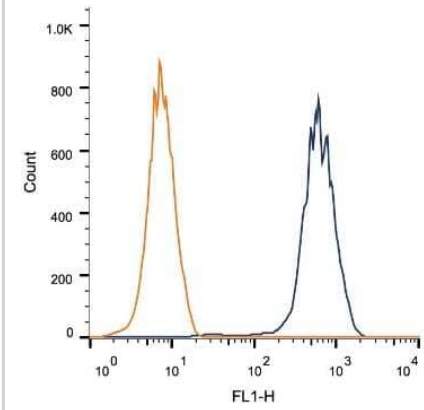


Flow Cytometry: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - An intracellular stain was performed on U937 cells with HGF LAMP-2/CD107b [H4B4] Antibody NBP2-22217AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Simple Western: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - Lane view shows a specific band for LAMP-2/CD107b in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - Localization of TMEM135 to the mitochondria. The mitochondrial fraction isolated from the WT mouse brain show TMEM135 signals by immunoblotting. Following proteins were used as organelle markers: MFN2-mitochondria; LAMP2-lysosome; Lamin B1- nucleus; PDI-endoplasmic reticulum (ER). Image collected and cropped by CiteAb from the following publication (https://elifesciences.org/articles/19264), licensed under a CC-BY license.	
Western Blot: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - Representative pictures of effect of I/R on DNA damage, cell survival, and inflammation. Western blot analysis expression level of Cathepsin B (*P < 0.05) in total protein extract from RVA of I/R treated vs. control group. Image collected and cropped by CiteAb from the following publication (https://www.frontiersin.org/article/10.3389/fphar.2018.01034/full), licensed under a CC-BY license.	

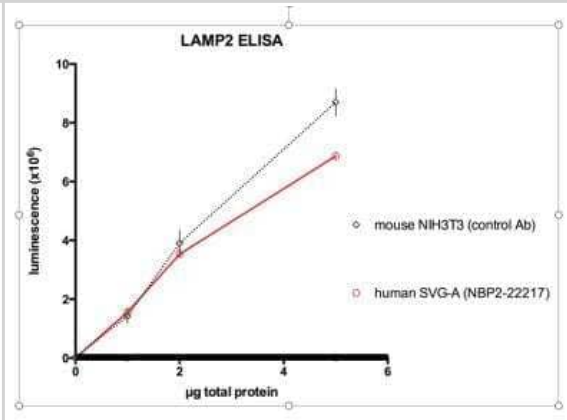
Immunocytochemistry/Immunofluorescence: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-LAMP-2/CD107b [NBP2-22217] at a 1:100 dilution overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



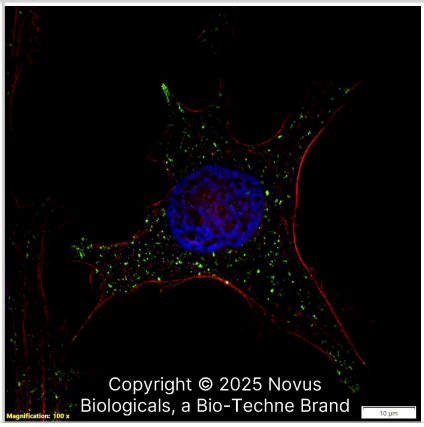
Flow Cytometry: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - Intracellular flow cytometric staining of 1 x 10^6 HEK-293 cells using anti-LAMP-2/CD107b (dark blue). Isotype control shown in orange. An antibody concentration of 1 ug/1x10^6 cells was used.



ELISA: LAMP-2/CD107b Antibody (H4B4) [NBP2-22217] - Anti-LAMP-2/CD107b was used to assess lysates from human SVG-A cells via ELISA, by loading the indicated mass of total protein per well. Antibodies tested were diluted 1:250, and used in conjunction with an HRP-conjugated secondary antibody. Signal was detected by luminescence. Image from verified customer review.



LAMP-2/CD107b (H4B4) was detected in immersion fixed HeLa human cervix adenocarcinoma cell line using Mouse anti-LAMP-2/CD107b (H4B4) Protein G-purified Monoclonal Antibody (Catalog # NBP2-22217) at 1.0 µg/mL overnight at 4C. Cells were stained using DyLight 488-conjugated Anti-Mouse IgG (H+L) Cross-Absorbed Secondary Antibody (green), and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Publications

Lee H, Nguyen Hoang AT, Lee SJ. Ginsenoside protopanaxadiol protects adult retinal pigment epithelial-19 cells from chloroquine by modulating autophagy and apoptosis PLOS ONE 2022-12-01 [PMID: 36454967]

Fathi A, Mathivanan S, Kong L et al. Chemically induced senescence in human stem cell-derived neurons promotes phenotypic presentation of neurodegeneration Aging Cell 2022-01-01 [PMID: 34953016]

Fang R, Ming T, Ng JPL et al. Ciliatoside A, isolated from Peristrophe japonica, inhibits HBsAg expression and cccDNA transcription by inducing autophagy Antiviral research 2022-12-07 [PMID: 36496141]

Chen C, Hashem S, Sharma J et al. Hematopoietic Stem Cell Gene Therapy Alleviates Disease Phenotypes in a Murine Model of Danon Disease Research Square 2022-08-19 (IHC-Fr, Mouse)

Price C, Jones S, Mihelcic M et al. Paradoxical Pro-inflammatory Responses by Human Macrophages to an Amoebae Host-Adapted Legionella Effector Cell Host Microbe 2020-03-30 [PMID: 32220647]

Pillai S, Mahmud I, Mahar R et al. Lipogenesis mediated by OGR1 regulates metabolic adaptation to acid stress in cancer cells via autophagy Cell reports 2022-05-10 [PMID: 35545051] (ICC/IF, Human)

Nanou A, Bourbouli M, Vetrano S et al. Endothelial Tpl2 regulates vascular barrier function via JNK-mediated degradation of claudin-5 promoting neuroinflammation or tumor metastasis Cell reports 2021-05-25 [PMID: 34038728] (IHC-P, Mouse)

Hirata H, Xu X, Nishioka K, et al. PMEPA1 and NEDD4 control the proton production of osteoclasts by regulating vesicular trafficking FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2021-02-01 [PMID: 33484199]

Yuniati L, Lauriola A, Gerritsen M et al. Ubiquitylation of the ER-Shaping Protein Lunapark via the CRL3KLHL12 Ubiquitin Ligase Complex Cell Rep 2020-05-19 [PMID: 32433973]

Fang EF, Hou Y, Palikaras K, et al Mitophagy inhibits amyloid-beta and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. Nat Neurosci. 2019 Feb 11 [PMID: 30742114] (EM, WB)

Pinazza Marica, Ghisi Margherita, Minuzzo Sonia et al. Histone deacetylase 6 controls Notch3 trafficking and degradation in T-cell acute lymphoblastic leukemia cells. Oncogene 2018-04-12 [PMID: 29643474] (IB, IF, Mouse, Human)

Ahmed M, Baumgartner R, Aldi S et al. Human serum albumin-based probes for molecular targeting of macrophage scavenger receptors Int J Nanomedicine 2019-05-21 [PMID: 31190821] (IF, Human)

More publications at http://www.novusbio.com/NBP2-22217

Procedures

Western Blot Protocol for LAMP-2/CD107b Antibody (NBP2-22217)
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.

Immunohistochemistry-Paraffin Protocol for LAMP-2/CD107b Antibody (NBP2-22217)
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.

Immunocytochemistry/ Immunofluorescence Protocol for LAMP-2/CD107b Antibody (NBP2-22217)
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.

Flow (Intracellular) Protocol for LAMP-2/CD107b Antibody (NBP2-22217)

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 1 mL 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:

Optional: Perform cell surface staining as described in the previous section.

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 5 minutes at 400 RCF.
5. Discard supernatant and re-suspend in 1 mL of staining buffer + 0.1% permeabilizer.
6. Stain each sample at 1 uL/ 1 x 106 cells of primary antibody or 1-3 uL/ 1 x 106 cells for directly conjugated antibodies. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
7. Following the primary/conjugate incubation, add 2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 5 minutes at 400 RCF.
8. Remove supernatant and re-suspend each sample in 2 mL staining buffer + 0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF.
9. If using a directly conjugated antibody, after the second wash, re-suspend cell pellet to a final volume of 500 uL per sample and proceed with flow analysis.

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Products Related to NBP2-22217

NB820-60568	Human Bladder Membrane Tissue Lysate (Adult Membrane Normal)
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)

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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