Product Datasheet

Lactate Dehydrogenase B Antibody - BSA Free
NBP2-53421

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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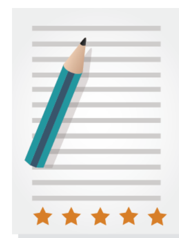


NBP2-53421

Lactate Dehydrogenase B 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	Immunogen affinity purified
Buffer	PBS

Product Description

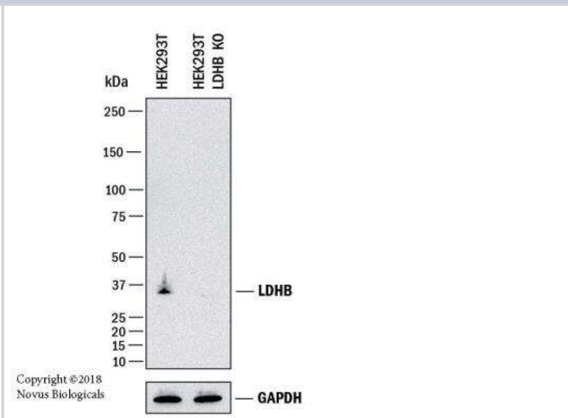
Description	Novus Biologicals Knockout (KO) Validated Rabbit Lactate Dehydrogenase B Antibody - BSA Free (NBP2-53421) is a polyclonal antibody validated for use in IHC, WB, Flow and ICC/IF. Anti-Lactate Dehydrogenase B Antibody: Cited in 5 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	3945
Gene Symbol	LDHB
Species	Human, Mouse, Rat
Immunogen	Partial recombinant human Lactate Dehydrogenase B protein (amino acids 1-93) [P07195]

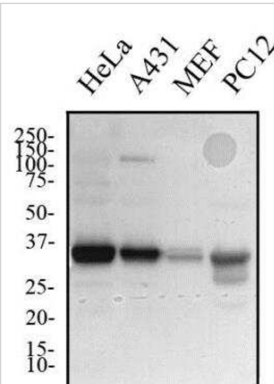
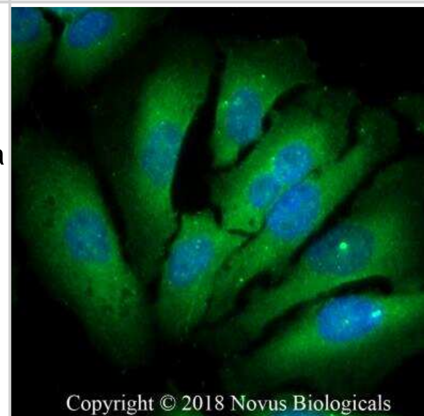
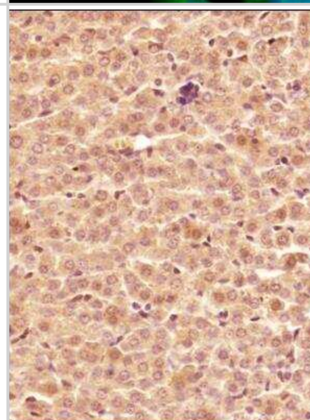
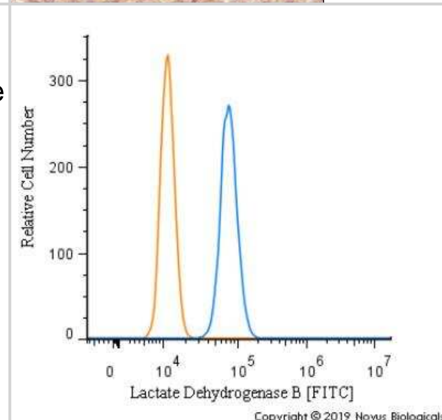
Product Application Details

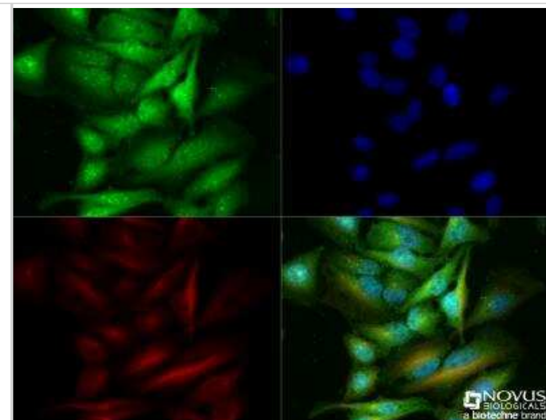
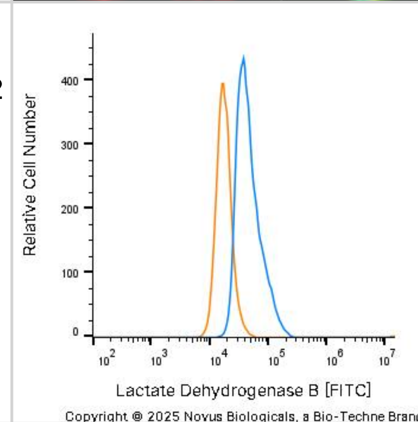
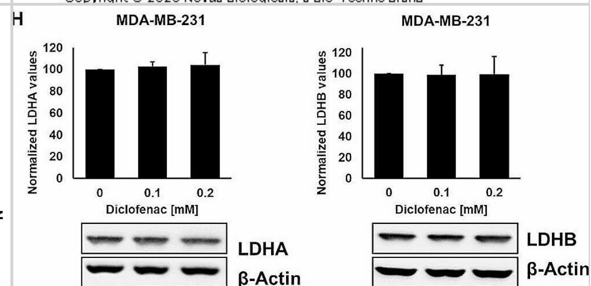
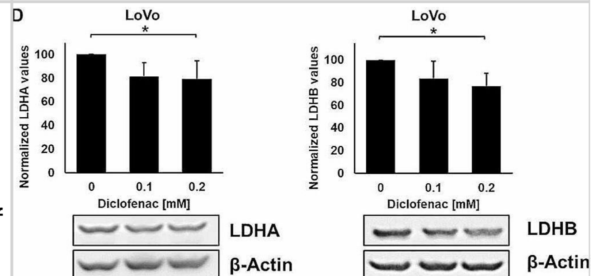
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Knockout Validated
Recommended Dilutions	Western Blot 1 ug/ml, Flow Cytometry 2-5 ug/ml, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence 5 ug/ml, Immunohistochemistry-Paraffin 1:400, Knockout Validated

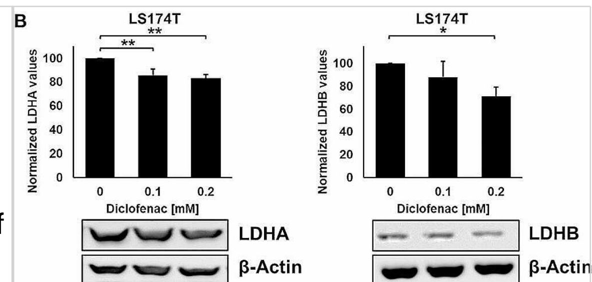
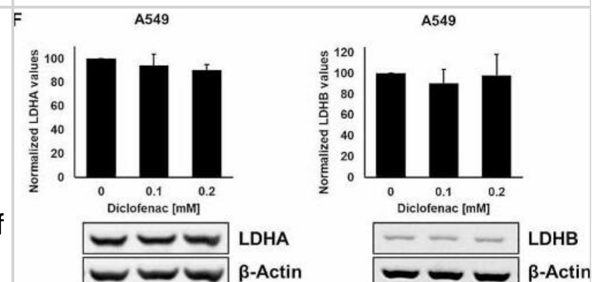
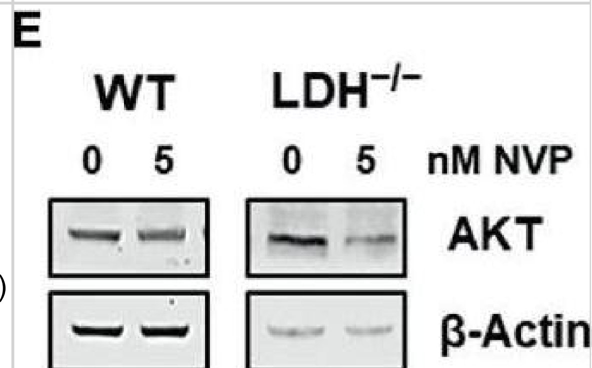
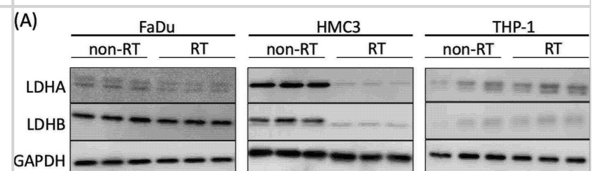
Images

Western Blot: Lactate Dehydrogenase B Antibody [NBP2-53421] - Western blot shows lysates of 293 human embryonic kidney parental cell line and LDHB knockout (KO) 293 cell line. PVDF membrane was probed with 1 ug/ml of Rabbit Anti-Human LDHB Polyclonal Antibody (NBP2-53421) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (HAF008). Specific band was detected for LDHB at approximately 35 kDa (as indicated) in the parental 293 cell line, but is not detectable in the knockout 293 cell line. This experiment was conducted under reducing conditions.



Western Blot: Lactate Dehydrogenase B Antibody [NBP2-53421] - Total protein from human HeLa and A431 cells, mouse MEF cells and rat PC12 cells 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 1.0 ug/ml anti-LDHB in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.	
Immunocytochemistry/Immunofluorescence: Lactate Dehydrogenase B Antibody [NBP2-53421] - HeLa 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 anti-LDHB at 2 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.	
Immunohistochemistry-Paraffin: Lactate Dehydrogenase B Antibody [NBP2-53421] - Analysis of a FFPE tissue section of mouse liver using Lactate Dehydrogenase B antibody at 1:400 dilution. The staining was developed using HRP-DAB detection method and the nuclei were counterstained with hematoxylin. The antibody generated a very specific staining in the hepatocytes.	
Flow Cytometry: Lactate Dehydrogenase B Antibody [NBP2-53421] - An intracellular stain was performed on SK-MEL-28 cells with Lactate Dehyrdrogenase B Antibody NBP2-53421F (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 FITC.	

Immunocytochemistry/Immunofluorescence: Lactate Dehydrogenase B Antibody [NBP2-53421] - 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-LDHB at a 1:200 dilution overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (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.	
An intracellular stain was performed on SJCRH30 human Rhabdomyosarcoma cell line with Rabbit anti-Lactate Dehydrogenase B Affinity-purified Polyclonal Antibody conjugated to FITC (Catalog # NBP2-53421F, blue histogram) or matched control antibody (NBP2-24892, orange histogram) at 10 µg/mL for 30 minutes at RT.	
LDH activity and expression levels in LS174T, LoVo, A549 and MDA-MB-231 cancer cells after diclofenac treatment. LDH activity assay of LS174T (A), LoVo (C), A549 (E) and MDA-MB-231 (G) cancer cells treated with diclofenac (0, 0.1 and 0.2 mM) for 48 h. Representative immunoblot showing the expression of LDHA and LDHB 48 h after diclofenac treatment in LS174T (B), LoVo (D), A549 (F) and MDA-MB231 (H) cancer cells. Quantification of the LDHA and LDHB signals of at least 3 independent experiments are shown in the bar charts above. The one way ANOVA test was used to evaluate significant differences (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001) Image collected and cropped by CiteAb from the following open publication (https://ro-journal.biomedcentral.com/articles/10.1186/s13014-024-02399-5), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
LDH activity and expression levels in LS174T, LoVo, A549 and MDA-MB-231 cancer cells after diclofenac treatment. LDH activity assay of LS174T (A), LoVo (C), A549 (E) and MDA-MB-231 (G) cancer cells treated with diclofenac (0, 0.1 and 0.2 mM) for 48 h. Representative immunoblot showing the expression of LDHA and LDHB 48 h after diclofenac treatment in LS174T (B), LoVo (D), A549 (F) and MDA-MB231 (H) cancer cells. Quantification of the LDHA and LDHB signals of at least 3 independent experiments are shown in the bar charts above. The one way ANOVA test was used to evaluate significant differences (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001) Image collected and cropped by CiteAb from the following open publication (https://ro-journal.biomedcentral.com/articles/10.1186/s13014-024-02399-5), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

LDH activity and expression levels in LS174T, LoVo, A549 and MDA-MB-231 cancer cells after diclofenac treatment. LDH activity assay of LS174T (A), LoVo (C), A549 (E) and MDA-MB-231 (G) cancer cells treated with diclofenac (0, 0.1 and 0.2 mM) for 48 h. Representative immunoblot showing the expression of LDHA and LDHB 48 h after diclofenac treatment in LS174T (B), LoVo (D), A549 (F) and MDA-MB231 (H) cancer cells. Quantification of the LDHA and LDHB signals of at least 3 independent experiments are shown in the bar charts above. The one way ANOVA test was used to evaluate significant differences (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001) Image collected and cropped by CiteAb from the following open publication (https://ro-journal.biomedcentral.com/articles/10.1186/s13014-024-02399-5), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
LDH activity and expression levels in LS174T, LoVo, A549 and MDA-MB-231 cancer cells after diclofenac treatment. LDH activity assay of LS174T (A), LoVo (C), A549 (E) and MDA-MB-231 (G) cancer cells treated with diclofenac (0, 0.1 and 0.2 mM) for 48 h. Representative immunoblot showing the expression of LDHA and LDHB 48 h after diclofenac treatment in LS174T (B), LoVo (D), A549 (F) and MDA-MB231 (H) cancer cells. Quantification of the LDHA and LDHB signals of at least 3 independent experiments are shown in the bar charts above. The one way ANOVA test was used to evaluate significant differences (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001) Image collected and cropped by CiteAb from the following open publication (https://ro-journal.biomedcentral.com/articles/10.1186/s13014-024-02399-5), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Hsp90 inhibition potentiates radiosensitivity in LS174T cells. (A) Colony forming assay of LS174T WT and LDH−/− cells after irradiation with 0, 0.5, 1 and 2 Gy (**p ≤ 0.01). Colony forming assay of LS174T WT (B) and LDH−/−(C) cells after treatment with a low concentration of NVP-AUY922 (5 nM) for 24 h and irradiation with 0, 0.5, 1 and 2 Gy (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001). (D) Comparison of WT and LDH−/− cells treated with a low dose of NVP-AUY922 (5 nM) (**p ≤ 0.01). (E) Representative immunoblot showing the expression of AKT and β-Actin in LS174T cells upon treatment with NVP-AUY922 (5 nM) for 24 h. (F, G) Membrane Hsp70 expression on B16F10 (F) and LS174T (G) cells treated with 100 nM NVP-AUY922 for 24 h, as determined by flow cytometry using the cmHsp70.1 mAb. The proportion of positively stained cells is shown (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/35463341), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Analysis of the expressions of LDHA and LDHB in the non-irradiated (non-RT) and irradiated (RT) cancer and immune cells. (A) Western blot analyses of LDHA and LDHB in FaDu, HMC3, and THP-1 cells. GAPDH was used as a loading control. (B) Comparison of the expressions of LDHA and LDHB between the non-irradiated and irradiated cells (* p < 0.05; ** p < 0.01). Note—LDH, lactate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/34436459), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Schwab M, Thunborg K, Azimzadeh O et al. Targeting Cancer Metabolism Breaks Radioresistance by Impairing the Stress Response Cancers (Basel) 2021-07-27 [PMID: 34359663]

Melissa Schwab, Ali Bashiri Dezfouli, Mohammad Khosravi, Bayan Alkotub, Lisa Bauer, Mohammad Javed Tahmasebi Birgani, Gabriele Multhoff The radiation- and chemo-sensitizing capacity of diclofenac can be predicted by a decreased lactate metabolism and stress response. Radiation oncology (London, England) 2024-01-18 [PMID: 38229111]

Schwab M, Dezfouli A, Khosravi M et al. The radiation- and chemo-sensitizing capacity of diclofenac can be predicted by a decreased lactate metabolism and stress response Research Square 2023-03-20 (WB)

Schwab M, Multhoff G A Low Membrane Hsp70 Expression in Tumor Cells With Impaired Lactate Metabolism Mediates Radiosensitization by NVP-AUY922 Frontiers in oncology [PMID: 35463341] (WB, Mouse)

Kumagai S, Koyama S, Itahashi K Et al. Lactic acid promotes PD-1 expression in regulatory T cells in highly glycolytic tumor microenvironments Cancer Cell 2022-01-29 [PMID: 35090594]

Details:
Citation using the PE version of this antibody.

Lai YC, Hsieh CY, Lu KY Et al. Monitoring Early Glycolytic Flux Alterations Following Radiotherapy in Cancer and Immune Cells: Hyperpolarized Carbon-13 Magnetic Resonance Imaging Study Metabolites 2021-08-06 [PMID: 34436459] (WB, Human)

Procedures

Western Blot Protocol for Lactate Dehydrogenase B Antibody (NBP2-53421)
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 Lactate Dehydrogenase B Antibody (NBP2-53421)
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 Lactate Dehydrogenase B Antibody (NBP2-53421)
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 Lactate Dehydrogenase B Antibody (NBP2-53421)
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 NBP2-53421

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
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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