# Product Datasheet

## BLIMP1/PRDM1 Antibody (3H2-E8) - BSA Free
## NB600-235SS

Unit Size: 0.025 ml

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

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**Reviews: 2 Publications: 52**

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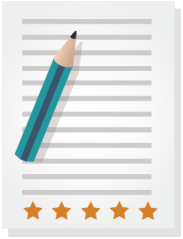
www.novusbio.com/NB600-235

Updated 10/23/2024 v.20.1





# NB600-235SS

BLIMP1/PRDM1 Antibody (3H2-E8) - BSA Free

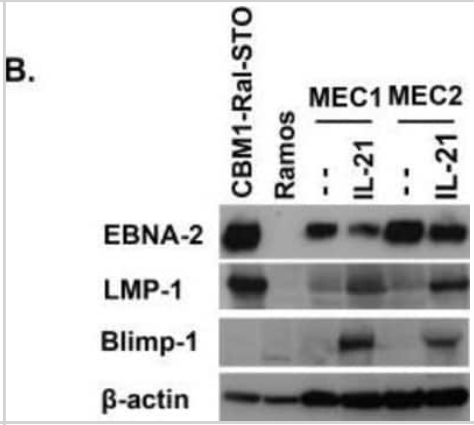
| Product Information | |
|---|---|
| **Unit Size** | 0.025 ml |
| **Concentration** | 1 mg/ml |
| **Storage** | Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles. |
| **Clonality** | Monoclonal |
| **Clone** | 3H2-E8 |
| **Preservative** | 0.02% Sodium Azide |
| **Isotype** | IgG1 |
| **Purity** | Protein G purified |
| **Buffer** | PBS |

| Product Description | |
|---|---|
| **Host** | Mouse |
| **Gene ID** | 639 |
| **Gene Symbol** | PRDM1 |
| **Species** | Human, Mouse, Porcine |
| **Reactivity Notes** | Porcine usage reported in customer reviews |
| **Marker** | Plasma Cell Marker |
| **Immunogen** | A fragment of mouse Blimp-1 corresponding to residues 199-409. [UniProt# Q60636] |

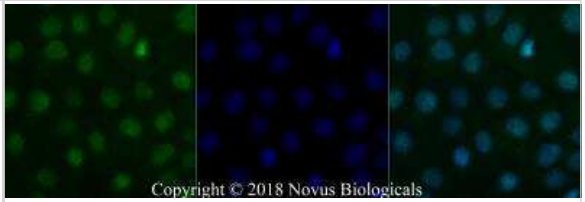
| Product Application Details | |
|---|---|
| **Applications** | Western Blot, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP) |
| **Recommended Dilutions** | Western Blot 1:1000, Flow Cytometry 8 ug/ml where cells are used at 2*10^6/mL, ELISA 1:100-1:2000, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunoprecipitation, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen reported in scientific literature, Chromatin Immunoprecipitation (ChIP) 1ug antibody/ 10 ug protein |
| **Application Notes** | By WB, this antibody recognizes a band at ~98 kDa and may recognize one at ~80 kDa (the beta form). Antigen retrieval is recommended (EDTA buffer, microwave) prior to IHC on paraffin tissues. This antibody demonstrates nuclear staining. For IHC and ICC/IF a dilution of 1:200 is recommended with tyramide amplification. |

## Images

Western Blot: BLIMP1/PRDM1 Antibody (3H2-E8) - BSA Free [NB600-235] - BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - The effect of IL-21 and CD40L exposure on MEC1 and MEC2 cells. Expression of EBNA-2 and LMP-1 in IL-21 treated cells. Expression of EBNA-2, LMP-1 and Blimp-1 by immunoblotting; positive control: CBM1-Ral-STO, negative control: Ramos. 1.5x105 cells were loaded in the control lanes and 5x105 were loaded in both untreated and IL-21 treated MEC1 and MEC2 lanes. Note low expression of EBNA-2 and high expression of LMP-1 after IL-21 treatment and induction of Blimp-1 after IL-21 treatment. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0106008), licensed under a CC-BY license.




Immunocytochemistry/Immunofluorescence: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-BLIMP1 (3H2-E8) at 10 ug/ml overnight at 4C and detected with an anti-mouse IgG Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.




Immunohistochemistry-Paraffin: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - Analysis using paraffin-embedded human tonsil tissue with BLIMP1/PRDM1 antibody at dilution of 1:50. Detection is completed through VC001 (DAB) and Counterstained by hematoxylin; labeling is predominantly in germinal centers.
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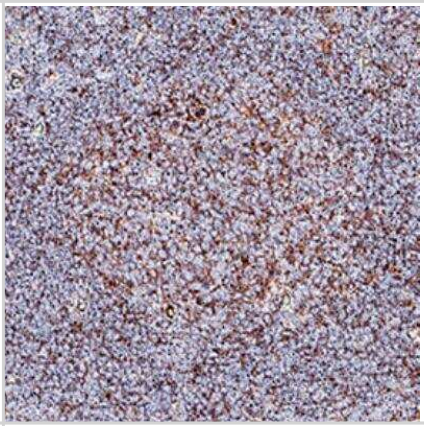
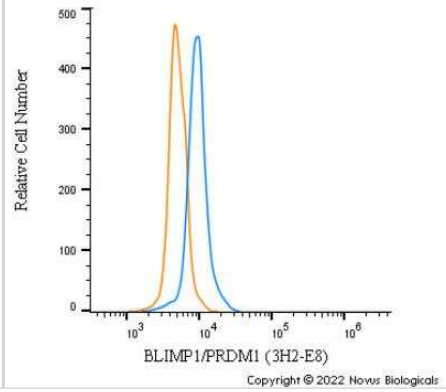


Flow Cytometry: BLIMP1/PRDM1 Antibody (3H2-E8) - BSA Free [NB600-235] - An intracellular stain was performed on A431 cells with BLIMP1/PRDM1 Antibody (3H2-E8) NB600-235 (blue) and a matched isotype control MAB002 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).



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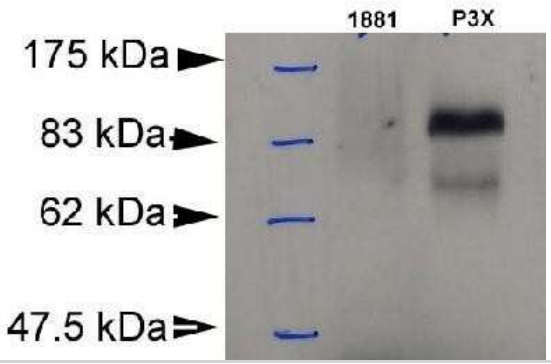
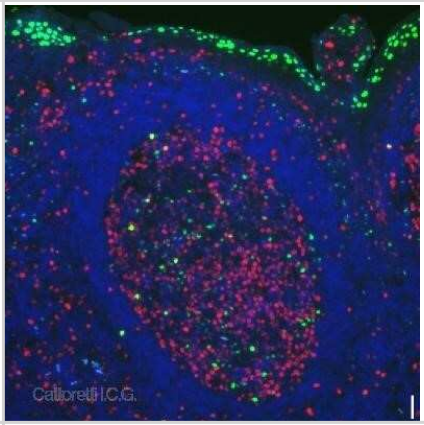
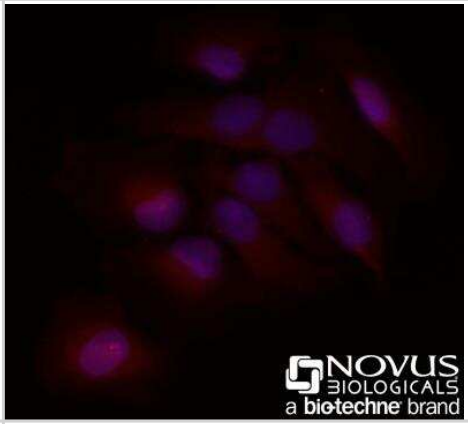
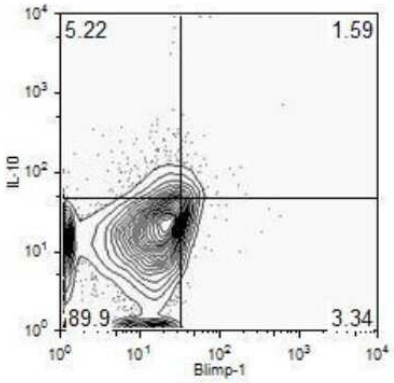
| Western Blot: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - Detection of Blimp-1 in murine plasmacytoma cell lysate (P3X) using NB 600-235. 1881: murine pre-B cell lysate (negative control). Photo courtesy of DA Savitsky, Columbia University. |  |
| Immunocytochemistry/Immunofluorescence: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - Double IF for Blimp-1 (green) and Ki-67 (proliferation, red) with DAPI counterstain, at 10x magnification (scale bar is 10um). Positive surface epithelium and centrocytes are labelled. Photo courtesy of Dr. Giorgio Cattoretti, Institute for Cancer Genetics, Columbia University, NY. |  |
| Immunocytochemistry/Immunofluorescence: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - HeLa 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 anti-BLIMP1/PRDM1 (3H2-E8) conjugated to DyLight 550 [NB600-235R] at 20ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective. |  |
| Flow Cytometry: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - IL-10+Blimp-1-, IL-10+Blimp-1+, IL-10-Blimp-1+ and IL-10-Blimp-1- cells, % total living single splenic CD19+ cells of 3 total mice (Tedder Lab; Duke Univ). |  |

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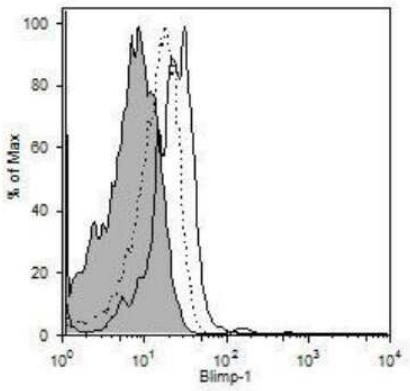
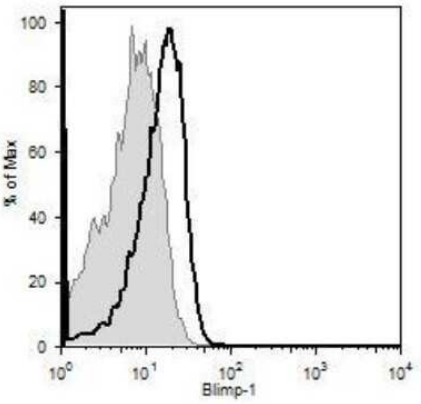
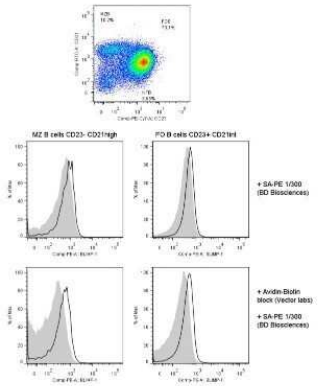
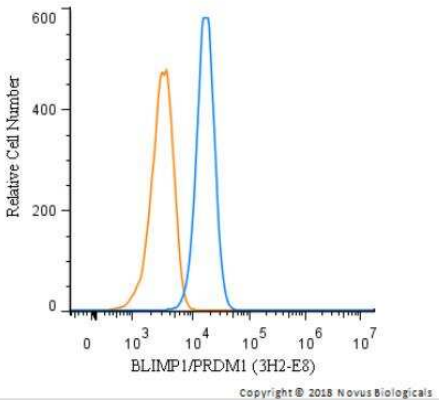
| Flow Cytometry: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - Blimp-1 expression by IL-10+ (black line), IL-10- (dotted line) or CD8+ (thin, shaded line) cells (Tedder Lab; Duke Univ) |  |
| Flow Cytometry: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - Blimp-1 expression by CD19+ (thick black line) or CD8+ (thin, shaded line). (Tedder Lab; Duke Univ). |  |
| Flow Cytometry: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - Analysis using the Biotin conjugate of NB600-235. Staining of Blimp-1 in mouse spleen. Image courtesy of product review by Branislav Krljanac. |  |
| Flow Cytometry: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - An intracellular stain was performed on U266 cells with BLIMP1/PRDM1 Antibody (3H2-E8) NB600-235 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and 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 mouse F(ab)2 IgG (H+L) APC-conjugated secondary antibody (F0101B, R&D Systems). |  |

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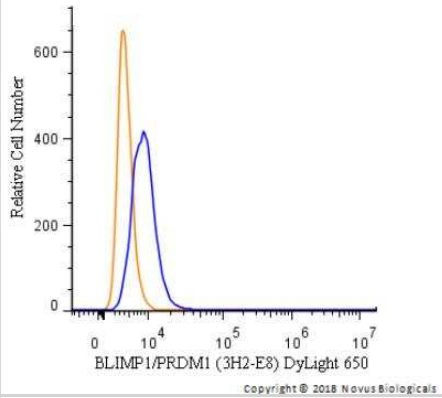
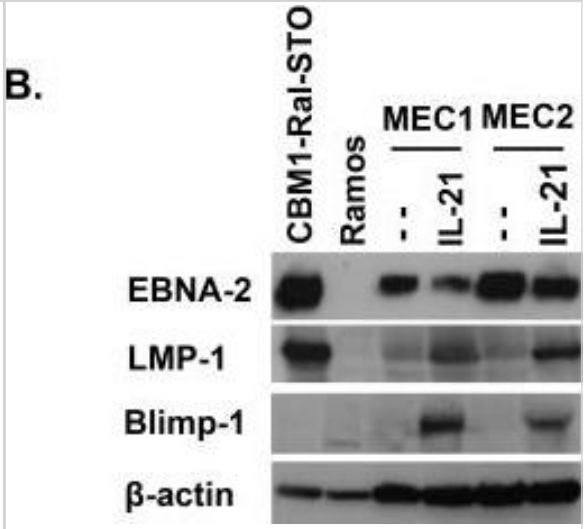
| Flow Cytometry: BLIMP1/PRDM1 Antibody (3H2-E8) [NB600-235] - An intracellular stain was performed on A431 cells with BLIMP1/PRDM1 [3H2-E8] Antibody NB600-235C (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to DyLight 650. |  |
| Western Blot: BLIMP1/PRDM1 Antibody (3H2-E8) - BSA Free [NB600-235] - The effect of IL-21 & CD40L exposure on MEC1 & MEC2 cells.Expression of EBNA-2 & LMP-1 in IL-21 treated cells (A, B). (A) Simultaneous immunofluorescence staining of EBNA-2 (Green) & LMP-1 (Red); magnification (×100), scale bar 25 µm. Note the downregulation of EBNA-2 & upregultion of LMP-1 after IL-21 treatment. (B) Expression of EBNA-2, LMP-1 & Blimp-1 by immunoblotting; positive control: CBM1-Ral-STO, negative control: Ramos. 1.5×105 cells were loaded in the control lanes & 5×105 were loaded in both untreated & IL-21 treated MEC1 & MEC2 lanes. Note low expression of EBNA-2 & high expression of LMP-1 after IL-21 treatment & induction of Blimp-1 after IL-21 treatment. (C) Activity of the W & C promoters that regulate EBNA-2 expression & LMP-1 mRNA expression by Q-PCR. Note the difference in EBNA-2 regulation; the MEC2 cell uses both Wp & Cp while in MEC1 only Wp is active. (D) Expression of EBNA-2 & LMP-1 in cells exposed to CD40L. Simultaneous immunofluorescence staining; for details see (A). Note: EBNA-2 & LMP-1 are downregulated by CD40L in both lines. (E) CD40L induced modulation of surface marker by FACS analysis. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0106008), licensed under a CC-BY license. Not internally tested by Novus Biologicals. |  |

## Publications


Xiong E, Popp O, Salomon C et al. A CRISPR/Cas9-mediated screen identifies determinants of early plasma cell differentiation Frontiers in Immunology 2023-01-05 [PMID: 36685499] (Western Blot, Block/Neutralize)

Hussaini M, Yeung C, Zhu X et al. PRDM1 expression levels in marginal zone lymphoma and lymphoplasmacytic lymphoma Int J Clin Exp Pathol 2020-01-23 [PMID: 31966717]

Iwanaga R, Truong BT, Lambert KA, et al. Loss of prdm1a accelerates melanoma onset and progression Mol Carcinog 2020-06-21 [PMID: 32562448]

Tan K, Wilkinson MF Regulation of both transcription and RNA turnover contribute to germline specification Nucleic acids research 2022-07-22 [PMID: 35776114] (ICC/IF)

Details:
Mouse ESCs were induced to differentiate into EpiLCs and PGCLCs

Nishikawa K, Seno S, Yoshihara T Et al. Osteoclasts adapt to physioxia perturbation through DNA demethylation EMBO reports 2021-10-18 [PMID: 34661337] (WB, Mouse)

Dong G, Li Y, Lee L et al. Genetic manipulation of primary human natural killer cells to investigate the functional and oncogenic roles of PRDM1 Haematologica 2020-07-30 [PMID: 32732362] (WB, Human)

Wang Y, Wang C, Cai X et al. IL-21 Stimulates the expression and activation of cell cycle regulators and promotes cell proliferation in EBV-positive diffuse large B cell lymphoma Sci Rep 2020-07-23 [PMID: 32704112] (WB, Human)

Tian Y, Seumois G, De-Oliveira-Pinto LM Molecular Signatures of Dengue Virus-Specific IL-10/IFN-gamma Co-producing CD4 T Cells and Their Association with Dengue Disease Cell Rep 2019-12-24 [PMID: 31875555] (Human)

Meer S, Perner Y, McAlpine E, Willem P Extra-oral plasmablastic lymphomas in a high HIV endemic area Histopathology Jul 30 2019 12:00AM [PMID: 31361906] Germinality does not necessarily define mAb expression and thermal stability. Appl Microbiol Biotechnol. 2019-09-01 [PMID: 31361906]

Details:
Citation using the FITC version of this antibody.

Cao, Y;Trillo-Tinoco, J;Sierra, RA;Anadon, C; ER stress-induced mediator C/EBP homologous protein thwarts effector Tcell activity in tumors through T-bet repression Nat Commun. [PMID: 30894532] (WB, Mouse)

Ye, Y;Liu, M;Tang, L;Du, F;Liu, Y;Hao, P;Fu, Q;Guo, Q;Yan, Q;Zhang, X;Bao, C; Iguratimod represses B cell terminal differentiation linked with the inhibition of PKC/EGR1 axis Arthritis Res. Ther. 2019-04-11 [PMID: 30971291] (FLOW, Human)

Wu L, Ehlin-Henriksson B, Zhou X et al. EBV provides survival factors to EBV+ DLBCL lines and modulates cytokine induced specific chemotaxis in EBV+ DLBCL Immunology 2017-07-12 [PMID: 28699226] (Human)


More publications at http://www.novusbio.com/NB600-235

## Procedures

**Western Blot Protocol for BLIMP1/PRDM1 Antibody (NB600-235)**
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 1% Non-fat milk 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 BLIMP1/PRDM1 Antibody (NB600-235)**
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 BLIMP1/PRDM1 Antibody (NB600-235)**

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.

**Immunohistochemistry-Paraffin Protocol for BLIMP1/PRDM1 Antibody (NB600-235)**
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.

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General: novus@novusbio.com


**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.

**For more information on our 100% guarantee, please visit www.novusbio.com/guarantee**

**Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB600-235**

**Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications**