www.novusbio.com
technical@novusbio.com
P: 303.760.1950
P: 888.506.6887
F: 303.730.1966

Human Plasmacytoid Dendritic Cell (pDC) Kit

Catalog Number: NBP2-29609 (10-25 tests)

Novus' Plasmacytoid Dendritic Cell (pDC) Kit is validated by Flow Cytometry for identifying pDC from human whole blood, freshly isolated peripheral blood mononuclear cells (PBMC) and frozen PBMC.

Note: Please read the entire protocol prior to beginning your experiments. The number of kit tests will vary from 10-25 depending on your experimental design. Following the protocol exactly will yield 10 full analysis tests, and hLMAX (NBP2-29608) will be the limiting reagent. However, each reagent vial has enough antibody for 25 tests. Additional kit components may be purchased separately to enable fl exibilityinexperimentaldesign.

Background: Plasmacytoid Dendritic Cells (pDC) are a subset of peripheral blood Dendritic Cells which play an important role in both innate and adaptive immune responses. pDC recognize viral components via Toll-like Receptors (TLR) including TLR7 and TLR9, and then rapidly produce Interferon-alpha (IFNα) as a primary anti-viral immune response. pDC also participate in antigen presentation, an important functional role which drives the adaptive immune response. Intense investigation has led to pDC identification with a unique set of markers by flow cytometry. pDC are usually present in relatively low frequency in peripheral blood. Direct identification of subsets of peripheral blood DCs has an advantage in analyzing changes in number or function of DC subsets during chronic viral infections or diseased patients, as examples.

Kit Contents:

Cat. No.	Component	Size
NBP2-29608	hLMAX: Human Lineage Marker Antibody Mix (human CD3, CD14, CD16, CD19, CD20, CD56, HLA-DR antibodies)*	10 ul/test, 300 ul
	CD123 AF647 (human CD123 Alexa Fluor 647 conjugate) antibody	5 ul/test, 125 ul
NBP2-24979	Mouse IgG1 Isotype control Alexa Fluor 647 conjugate antibody	5 ul/test, 125 ul
KC-136	Staining buffer (1X)	1 x 60 ml
KC-137	Fixation buffer (1X)	60 ml

* CD3, CD14, CD16, CD19, CD20 and CD56 are conjugated to FITC, HLA-DR is conjugated to PerCP-Cy5.5.

Storage Conditions: Store at 4°C, do not freeze; conjugated antibody is light-sensitive

Experimental Design

Tube #	Cells ($1x10^6$)	hLMAX	Isotype AF647	CD123 AF647
1	✔			
2	✔	✔	✔	
3	✔	✔		✔

Before you begin: *It is recommended that users follow the protocol provided for the best results with this kit.*

Single color (AF647, FITC, PE, PerCP-Cy5.5) stained samples are recommended as compensation controls for flow cytometric analysis. Reagents for compensation controls are not provided in this kit.

All staining and incubation steps should be done using light protected procedures. Commonly this is done by covering the sample racks or ice bucket with tin foil.

Protocol for staining PBMC or whole blood

1. Determine the number of cells required for staining which include cells for staining as well as cells for unstained control. Note: $1x10^6$ cells per each staining sample is generally needed.
2. Add 100 ul of blood or PBMC suspension ($1x10^6$ cells in 100 ul) to each of four labeled tubes.
3. Add 10 ul of hLMAX (NBP2-29608) to all tubes except Tube #1.
4. Add 5 ul of Mouse IgG1 AF647 conjugate (NBP2-24979) to Tube #2
5. Add 5 ul of CD123 AF647 conjugate to Tubes #3.

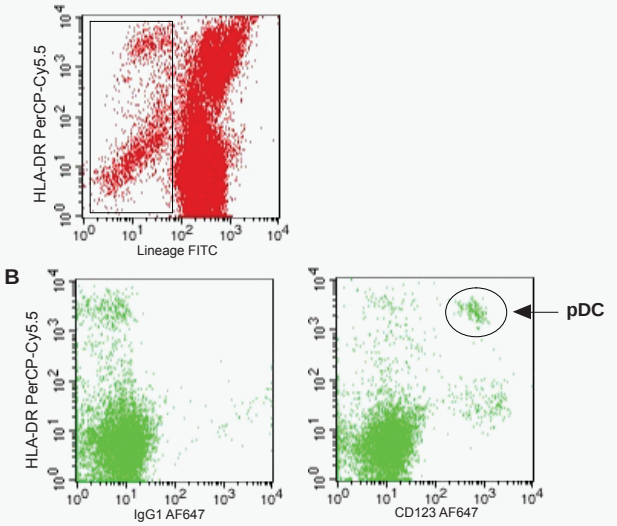
Note: All subsequent Steps apply to all tubes unless otherwise noted.

6. a. For PBMC, incubate for 20 min on ice.
   b. For whole blood, incubate for 20 min at room temperature (RT). After incubation, add 2 ml of RBC lysis buffer (sold sepa-rately, Cat. No. NBP2-29442) and continue incubating for another 10 min at RT.

Note: All subsequent Steps should be performed in the cold.

7. Add 2 ml of cold 1X Staining buffer (KC-136).
8. Centrifuge for 10 min at 1200 RPM.
9. Aspirate/decant the supernatant being careful not to lose the cells.
10. Repeat Steps 7-9 to wash.
11. Resuspend the pellet in 300 ul of 1X Staining buffer (KC-136) and analyze by flow cytometry. **Note:** If not analyzing on the same day, resuspend cells in 1X Fixation buffer and store overnight at 4°C. The Fixation buffer can be removed and the cells prepared for analysis by repeating Step 7-9 and adding 300 ul of Staining buffer to each tube.

Flow cytometric analysis using the NBP2-29610 pDC Kit A



A. Cell surface staining of fresh PBMC with hLMAX (NBP2-29608). Lineage negative and HLA-DR positive cells were gated.
B. Lineage negative and HLA-DR positive cells were analyzed for Mouse IgG1 (NBP2-24979, left panel) or CD123 (right panel). The pDC population (CD123 positive and HLA-DR high) was gated.

Related Products:

1. Red Blood Cell Lysis Buffer (Cat # NBP2-29442)
2. Human Lineage Marker Antibody Cocktail (hLMAX) (Cat # NBP2-29608)
3. Plasmacytoid Dendritic Cell (pDC) Identification Kit (Cat # NBP2-29609)
4. TLR9 PE Monoclonal Antibody (Cat # NBP2-24907)
5. IC-Flow (Intracellular Staining Flow Assay) Kit (Cat # NBP2-29450)
6. CS-Flow (Cell Surface Staining Flow Assay) Kit (Cat # NBP2-29481)
7. Intracellular Toll-like Receptor Staining Flow Kit (Cat # NBP2-26248)
8. Cell Surface Toll-like Receptor Staining Flow Kit (Cat # NBP2-26247)

References:

1. Kadowaki, N., S. Antonenko, J.Y. Lau, and Y.J. Liu. 2000. *J Exp Med* 192:219–226
2. Blom, B., Ho, S., Antonenko, S., and Liu, Y. J. 2000 *J Exp Med* 192, 1785–96
3. Dzionek A, Fuchs A, Schmidt P, et al. 2000. *J Immunol* 165:6037–6046
4. N. Kadowaki, S. Ho, S. Antonenko, R.W. Malefyt, R.A. Kastelein, F. Bazan, Y.J. Liu, *J Exp Med* 194 (2001) 863–869

Caution: *Fixation buffer contains 0.04% paraformaldehyde and 0.02% sodium azide. Staining buffer contains 0.02% sodium azide and antibodies contain 0.05 % sodium azide. Use caution when handling. All the materials included in this kit should be handled as hazardous materials and be disposed as required by your institution.*

For Research Use Only