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Human Plasmacytoid Dendritic Cell (pDC)/TLR9 Kit

Catalog Number: NBP2-29610 (10-25 tests)

Novus' Plasmacytoid Dendritic Cell (pDC)/TLR9 Kit is validated by Flow Cytometry for identifying human TLR9 expression in 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. For example, the TLR9 antibody is used at 5 ul/test and the reagent vial contains 125 ul which yields 25 tests. Additional kit components may be purchased separately to enable flexibility in experimental design.

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
NBP2-24907	TLR9 PE (human TLR9 PE conjugate) antibody	5 ul/test, 125 ul
NBP2-24979	Mouse IgG1 Isotype control Alexa Fluor 647 conjugate antibody	5 ul/test, 125 ul
NBP2-24976	Mouse IgG1 Isotype control PE conjugate antibody	5 ul/test, 125 ul
KC-136	Staining buffer (1X)	2 x 60 ml
KC-137	Fixation buffer (1X)	60 ml
KC-138	Permeabilization buffer (10X)	2 x 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	Isotype PE	TLR9 PE
1	✔					
2	✔	✔	✔			
3	✔	✔		✔	✔	
4	✔	✔		✔		✔

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

Permeabilization buffer (KC-138) is supplied as a 10X solution. Dilute with deionized water to its final 1X working concentration immediately prior to use (example: 1 ml of 10X Permeabilization buffer to 9 ml of deionized water).

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 and #4.

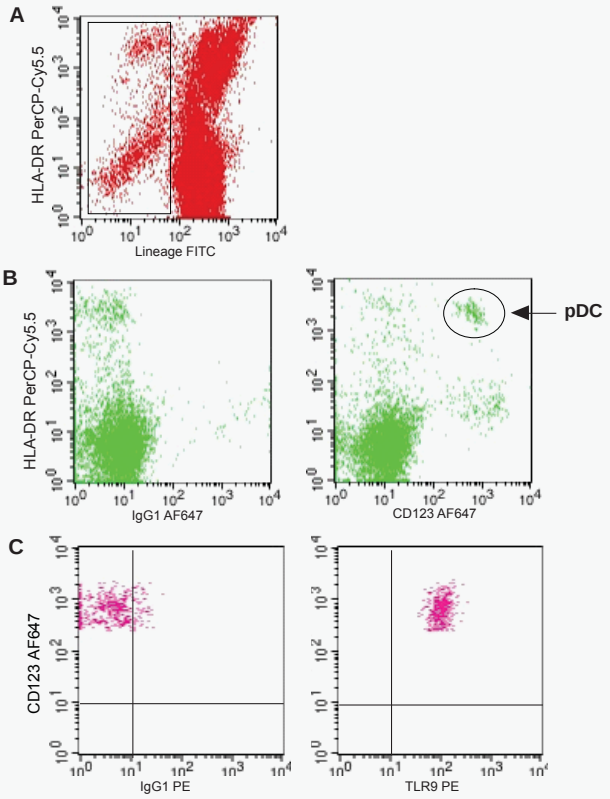
Note: All subsequent Steps apply to all tubes unless otherwise noted.

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, # 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 into the residual buffer (~100 ul) and add 300 ul of 1X Fixation buffer (KC-137).
12. Pulse vortex and incubate on ice for 20-25 min.
13. Add 2 ml 1X Permeabilization buffer (KC-138).
14. Centrifuge for 10 min at 1200 RPM.
15. Resuspend the pellet into the residual buffer (~100 ul) and incubate with 1 ml 1X Permeabilization buffer (KC-138) on ice for 10 min.

Flow cytometric analysis using the NBP2-29610 pDC/TLR9 Kit



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.
C. pDCs were analyzed for Mouse IgG1 (NBP2-24976, left panel) or TLR9 (NBP2-24907, right panel). The pDC were TLR9 positive.

16. Add 2 ml 1X Permeabilization buffer (KC-138), centrifuge for 10 min for 1200 RPM and repeat.
17. Resuspend the pellet into the residual buffer (~100 ul) by gentle vortex.
18. Add 5 ul of Mouse IgG1 PE conjugate (NBP2-24976) to Tube #3.
19. Add 5 ul of TLR9 PE conjugate (NBP2-24907) to Tube #4.
20. Mix the contents by gentle vortex and incubate on ice for 25 min.
21. Wash the stained cells with 2 ml 1X Permeabilization buffer (KC-138).
22. Centrifuge for 10 min at 1200 RPM and decant (aspirate).
23. Resuspend the pellet in 300 ul of 1X Staining buffer (KC-136) and analyze by flow cytometry. Samples can be stored overnight in the dark at 2-8°C prior to analysis.

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) IdentificationKit (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

Product Citation:

Tonsillar TLR9 expression and efficacyoftonsillectomywithsteroidpulsetherapyinIgAnephropathypatients. Sato D, Y Suzuki, T Kano, H Suzuki, J Matsuoka, H Yokoi, S Horikoshi, K Ikeda, Y Tomino. Nephrol Dial Transplant 0:1-8 (2011). **Novus product cited: Plasmacytoid Dendritic Cell (pDC)/TLR9 Kit (NBP2-29610). Flow (cell surface & intracellular): Primary human tonsillar B cells, Fig 3B.**

Caution: *Fixation buffer contains 0.04% paraformaldehyde and 0.02% sodium azide. The 10X Permeabilization buffer contains 1% saponin and 0.1% 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.*

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