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Live Cell Discrimination and Simultaneous Measurement of Phosphorylation and Cell Surface Markers in Thawed and Activated Human PBMCs Using BD Horizon™ Fixable Viability Stain 450, BD Cytofix™ Fixation Buffer, and BD Phosflow™ Perm Buffer III

Reagents Used

BD Phosflow™ Mouse Anti-Stat3(pY705) Alexa Fluor® 647, Clone 4/P-STAT3 (Cat. No. 557815)

BD Phosflow™ Mouse Anti-Stat5 (pY694) Alexa Fluor® 647, Clone 47/Stat5(pY694) (Cat. No. 612598)

BD Pharmingen™ Mouse Anti-Human CD3 PE, Clone UCHT1 (Cat. No. 555333)

BD Pharmingen™ Mouse Anti-Human CD4 PerCP-Cy™5.5, Clone L200 (Cat. No. 552838)

BD Horizon™ Fixable Viability Stain 450 (FVS450) (Cat. No. 562247)

BD Cytofix™ Fixation Buffer (Cat. No. 554655)

BD Phosflow™ Perm Buffer III (Cat. No. 558050)

BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656)

BD Pharmingen™ Recombinant Human IL-2 (Cat. No. 554603)

BD Pharmingen™ Recombinant Human IL-6 (Cat. No. 550071)

Cell culture grade dimethyl sulfoxide (DMSO) (Sigma Cat. No. D2650)

1X Dulbecco's phosphate buffered saline (DPBS) (Life Technologies)

Procedural Notes

Detailed procedures for peripheral blood mononuclear cell (PBMC) preparation, reagent preparation, activation, fixation, and viability staining are described in the TDS for BD Horizon FVS450.

http://www.bdbiosciences.com/ds//pm/tds/562247.pdf

Detailed procedures for peripheral blood mononuclear cell (PBMC) preparation, activation, fixation, permeabilization, and staining are described in Protocol III of the *BD Phosflow™ Protocols for Human PBMCs*.

http://static.bdbiosciences.com/documents/Phosflow_Protocol_for_Human_PBMCs.pdf

Preparation and Storage of FVS450

Bring FVS450 dye powder and fresh DMSO to room temperature, and add 400 µL of DMSO to the FVS450 dye. Vortex the solution until fully dissolved. The reconstituted FVS450 dye solution can be stored at -20°C and used for up to four freeze-thaw cycles. Aliquots can be made and stored at -20°C for up to 40 days. Discard the dye solution after 40 days after reconstitution.

Cells

Human PBMCs are freshly prepared, frozen, and then thawed for activation.

Stimulation

Cells are thawed, washed, and resuspended in protein-free and azide-free 1X DPBS, and then activated for Stat3(pY705) expression (with 100 ng/mL of recombinant human IL-6), or for Stat5(pY694) expression (with 100 ng/mL of recombinant human IL-2) for 15 minutes in a 37°C water bath.

Viability Staining for Live Cell Discrimination

FVS450 is added to the activated cells for the last 7 minutes of stimulation, at 1 µL of FVS450 per mL of cells. The cells are incubated for 7 minutes at 37°C protected from light. Cells are washed twice with Stain Buffer (FBS) and suspended at 1 x 10^6 per test (100 µL).

Fixation and Permeabilization

Cells are fixed using BD Cytofix Fixation Buffer and permeabilized using BD Phosflow Perm Buffer III as described in Protocol III of the BD Phosflow™ Protocols for Human PBMCs.

http://static.bdbiosciences.com/documents/Phosflow_Protocol_for_Human_PBMCs.pdf

Flow Cytometric Analysis of Stained Cell Samples

Flow cytometric analysis of the samples and controls can be performed on a BD LSRFortessa™ flow cytometer equipped with three lasers: a 488-nm blue laser, a 633-nm red laser, and a 405-nm violet laser. For each cell sample, about 5,000 events are collected in the lymphocyte gate. Prior to sample collection, fluorescence compensation settings are established using single-color BD™ CompBead control samples and the BD FACSDiva™ software compensation procedure.

Example 1

Multicolor flow cytometric analysis of phosphorylated Stat3(pY705) expression by "viable" activated human PBMCs.

http://www.bdbiosciences.com/ds//pm/tds/562247.pdf

Example 2

Multicolor flow cytometric analysis of phosphorylated Stat5(pY694) expression by "viable" activated human PBMCs.

http://www.bdbiosciences.com/ds//pm/others/23-13871.pdf

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