

Materials List for:

Multicolor Flow Cytometry Analyses of Cellular Immune Response in Rhesus Macaques

Hong He¹, Amy N. Courtney¹, Eric Wieder², K. Jagannadha Sastry¹

¹Department of Immunology, MD Anderson Cancer Center - University of Texas

²Department of Medicine, University of Miami

URL: <https://www.jove.com/video/1743>

DOI: [doi:10.3791/1743](https://doi.org/10.3791/1743)

Materials

<p>Solution and Buffers</p> <ul style="list-style-type: none"> • Ficoll-Hypaque (Histopaquen-1077, Sigma-Aldrich, St. Louis, MO) • RPMI-1640 medium (HyClone laboratories, Logan, UT) • Fetal Bovine Serum (FBS), (HyClone laboratories, Logan, UT) • L-glutamine (Sigma-Aldrich; St. Louis, MO) • Penicillin/streptomycin (Invitrogen; Carlsbad, CA) • Trypan blue reagent (Sigma-Aldrich; St. Louis, MO) • 50 mM Tris, PH 8.6; filtered and store at 4 °C • Dulbecco's PBS (DPBS, Ca2/Mg2-free; Life technologies, Rockville, MD) • Flow wash buffer: DPBS supplement with 2% FBS, store at 4 °C • BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit (BD Biosciences; San Jose, CA)
<p>Activation agents</p> <ul style="list-style-type: none"> • Phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich, St. Louis, MO) • Ionomycin (Sigma-Aldrich, St. Louis, MO) • Costimulatory mAb CD49d, clone 9F10 (BD Biosciences; San Jose, CA) • Affinity purified antibody F (ab)2 fragments of Goat Anti-Mouse IgG (H + L) (Kierkegaard and Perry Laboratory, Gaithersburg, MD) • Specific activation agents could include peptide pools, single peptide, or whole protein
<p>Cytokine secretion inhibitors</p> <ul style="list-style-type: none"> • Brefedin A (BFA, Sigma-Aldrich, St. Louis, MO) • Golgi Plug can be used alternatively (BD Biosciences; San Jose, CA)
<p>Antibodies</p> <ul style="list-style-type: none"> • Live/dead fixable aqua fluorescent stain kit (Invitrogen; Carlsbad, CA) • CD3 PE-Cy7 (SP34-2), (BD Biosciences; San Jose, CA) • CD4 pacific blue (OKT4), (eBiosciences (San Diego, CA) • CD8 Alexa700 (RPA-T8), (BD Biosciences; San Jose, CA) • CD28 PerCP-cy5.5 (L293), (BD Biosciences; San Jose, CA) • CD95 APC (DX2), (BD Biosciences; San Jose, CA) • IFN- FITC (B27), (BD Biosciences; San Jose, CA) • IL-2 PE (MQ1-17H12), (BD Biosciences; San Jose, CA)