Protocol for LPS Activation of MAPK Signaling in Whole Blood Samples

I. Procedure

1. Using universal precautions, pipette 100ul of blood into the bottom of 12X75 mm tubes
2. Remove blood from side of tube with cotton swab to eliminate potential contamination of the sample with unfixed cells.
3. Add 100 ng LPS to activation tubes (or equal volume of PBS to control tubes). Place tubes in 37 deg C water bath.
4. After 10 min incubation, remove first tube from incubator and pipette 65ul 10% formaldehyde into tube
5. Vortex well, and place tubes in rack, incubate at room temperature
6. After exactly 10 minutes incubation with formaldehyde, pipette 1 ml Lyse/Permeabilization Buffer (pre-warmed to 37 deg C) into each tube, vortex vigorously and return to rack. Incubate at room temperature for 15 minutes
7. Add 2 ml cold wash buffer (4^0 C) to each tube. Centrifuge all tubes at 1000 X G for 3 minutes. Remove tubes from centrifuge and aspirate as much supernate fluid as possible.
8. Resuspend pellet w 1 ml cold (4^0 C) 50% MeOH in PBS. Centrifuge and wash 2 X with 2 ml cold wash buffer. After final centrifugation, aspirate as much supernate as possible.
9. Add antibodies and cold Wash Buffer to a final volume of 100ul. Incubate at room temperature for 30 minutes.
10. Add 2 ml cold Wash buffer, mix and centrifuge. Resuspend cells in 1 ml Wash Buffer, vortex and analyze on the flow cytometer.

II. Reagents:

- anticoagulated blood (e.g. lithium heparin, or K_2 EDTA)
- 12 X 75 mm polypropylene tubes
- 10% Formaldehyde (methanol free) l liter, Polysciences, Inc., Cat # 04018
  Formaldehyde is stored at room temperature in the dark and used within 6 months
- Triton X-100, 10% aqueous solution, 6 ampoules x 10ml, Pierce, Cat # 28314
- Lippopolysaccharides (LPS) (from E coli 0127:B8), Sigma, Cat # L4516 (dilute to 10 ug/ml in PBS)
- BSA (Sigma 30% Cat # A7284)
- Fetal Bovine Serum (Hyclone Cat # SH30088-02)
- Phosphate Buffered Saline (PBS) calcium and magnesium free, Gibco, Cat # 20012 (or equivalent)
- Sodium Azide (NaN_3), USB Corp., Cat # 21610 (or equivalent)
- 100% Methanol, reagent grade (store at minus 20 deg C.)
- Cotton-tipped swabs
- Vortex mixer
- 37 deg C bath

Antibodies used in this lab:
CD14-PECy7 (clone RMO52), Beckman Coulter Inc., Cat # A22331

P-p38 MAPK-Alexa 488 (T180/Y182) (clone 36/p38), Becton Dickinson Cat # 612594 (or Cell Signaling Technology Clone 28B10, Cat # 4551)

P-SAPK/JNK-PE (T183/Y185) (clone 69) Cell Signaling Technologies (this is a custom conjugate of CST Cat # 9255)

P-ERK-Alexa 647 (T202/Y204) (also named P-p44/42 MAPK) (clone E10), Beckman Coulter Inc., Cat. # A24062 (or Becton Dickinson Clone 20A, Cat #612593)

Lysis/Permeabilization Buffer

Dilute 114ul Triton X-100 10% solution to 10 ml with PBS
Store this 0.114% Triton X-100 solution at room temperature in the dark.
Pre-heat Lyse/Permeabilization Buffer to 37 deg C immediately prior to use.

Wash Buffer

PBS (w/o Ca++/Mg++) with 4% BSA, or 4 % FBS, sterile filter (0.22 u filter), store at 4 deg C.

Courtesy of Vince Shankey, BCI, Inc.