

## **ImageStream® Monocyte Shape Change**

### **Whole Blood Monocyte Shape Change**

#### **Experimental Procedures**

##### **Samples**

Single fluorescent color control samples – use 150 µl whole blood for single color control.

Experimental samples – use 150 µl whole blood and stain according to following protocol.

##### **Materials**

01. MCP-1 10ug (Sigma #M6667)
02. anti-CD45 FITC (BD #340664)
03. Wash Buffer (Phosphate buffered saline without  $\text{Ca}^{2+}$ / $\text{Mg}^{2+}$  (PBS) + 2% FBS)
04. Fix Buffer (1% Formaldehyde in PBS)
05. Fix-FACS-lyse (6:3:1 mix of diH<sub>2</sub>O, 10% formaldehyde (Polysciences # 04018), FACS-lyse (BD #349202))
06. 15 cc microcentrifuge tubes (BD Falcon #352095)
07. 0.6 mL siliconized microcentrifuge tubes (Sigma # T4691)
08. Lavender Top Vacutainer
09. Holder
10. Needle

##### **Protocol**

01. Prepare 10ug/ml MCP-1 by adding 1ml of PBS 0.1%BSA to 10ug of MCP-1. Store -20C.
02. Draw blood in anticoagulant EDTA (lavender top) vacutainer tubes. Assume  $5 \times 10^6$  cells/ ml.
03. Gently aliquot 150 uL blood to 2ml eppendorf tube with wide bore tip per sample.
04. Add CD14 FITC (1:20) if needed.
05. Incubate 37C, 30 minutes (to decrease background shapechange)
06. Add MCP-1 and incubate 20min at 37C. Positive control = 0.1ug MCP-1 or 1.5ul into 150ul of blood for 20min at 37C.
07. Add 1.5 mL Fix-FACS-lyse 10' RT (or 10x actual sample volume)
08. Centrifuge 300xg 10'.
09. Add 1.5 mL wash buffer and Centrifuge 300xg 10'.
10. Resuspend in 100 uL wash buffer and stain with CD45 FITC (1:20) 10' RT.
11. Add 1 mL wash buffer and Centrifuge 300xg 10'
12. Resuspend 60 µL in fix buffer and run on the ImageStream.