



TransFix[®] Bone Marrow Procedure

Sample preparation for flow cytometry

1. Place 3ml of aspirated bone marrow into EDTA blood collection tube and mix by inversion 5-10 times
2. Add 0.2ml of TransFix[®] 1ml of bone marrow aspirate
3. Store at 2°C to 8°C

Flow Cytometry Preparation Procedure

1. Place a 2ml aliquot of TransFix[®] treated bone marrow in a 15ml conical tube
2. Slowly add 5ml of Hank's solution and mix gently by inversion
3. Centrifuge for 5 minutes at 2000 rpm and aspirate supernatant to waste
4. Resuspend cell pellet in 13ml of ammonium chloride and mix gently by inversion
5. Place in the dark for 5 minutes to lyse the red blood cells
6. Centrifuge for 5 minutes at 2000 rpm and aspirate supernatant to waste
7. Add 13ml of Hank's solution and mix gently by inversion
8. Centrifuge for 5 minutes at 2000 rpm and aspirate supernatant to waste
9. Add 1ml of 2% new born bovine serum and vortex
10. Perform a cell viability count and adjust the cell concentration to 2×10^7 cells/ml

Flow cytometric analysis

1. Instrument Procedure. Follow instrument manufacturers instructions for instrument alignment and sample analysis
2. Fluorescent antibody procedure. Use fluorescent antibodies according to manufacturers instructions for patient and control samples
3. RBC lysing procedure. Follow manufacturers lysing reagent instructions