

# Protocol for Cell Sorting:

## Reagents:

Lympholyte-Poly, from Cedar Lane Labs

RBC lysis buffer (Roche),

10% human serum in PBS

Wash Buffer: Cold PBS+2% FBS

## Aim:

To obtain tissue specific gene expression profiles from T-cells, monocytes, neutrophils and Natural Killer Cells , that have been sorted from whole blood.

Blood was collected into 3 ACD tubes from a healthy donor. Total white cells were separated from most of the RBC using a density gradient column ( Lympholyte-Poly), according to manufacturer's instructions. Any remaining RBCs were removed with RBC lysis buffer (Roche), according to manufacturer's instruction. This heterogenous population should contain Peripheral Blood Mononuclear cells(including T and B lymphocytes and Natural Killer cells),granulocytes(including neutrophils) and monocytes. After blocking with 10% human serum, the cells were stained for 15 mins, on ice, with CD3-APC(T-cells); CD66B-FITC(neutrophils); CD64-CyC(monocytes); CD-56 PE(NK-cells). Excess stain was washed with cold PBS+2% FBS and cells were sorted out into 4 pure populations of T-cells, neutrophiles, monocytes and NK-cells. Four color, 4-way sort was done using FacsVantage SE Turbo with FACS Diva option(BD Biosciences). A post-sort purity analysis was done. RNA was isolated from the 4 cell types using Qiagen RNeasy kit. Labelled cRNA probes were made according to protocol from Affymetrix and these probes were used to obtain gene expression profiles using Affymetrix Microarray technology.

## Tubes for staining:

1. unstained
2. Isotype controls (5ul each)
3. CD3-FITC (about  $10^6$  cells)
4. CD3-PE (about  $10^6$  cells)
5. CD8-CyC (about  $10^6$  cells)
6. CD3-APC (about  $10^6$  cells)
7. CD66B-FITC(5ul/ $10^6$  cells), CD56-PE (5ul/ $10^6$  cells), CD64-CyC(5ul/ $10^6$  cells), CD3-APC (2.5 ul / $10^6$ cells).

## Notes:

Presort analysis has to be done.

Template od acquisition should be saved.

Post sort purity analysis should be done to ascertain the purity of the samples.

Remind Greg or Janet to set up the cooling system and also specify if sterile conditions are required.