# Cell sorting protocol

## Dead cell removal kit

1. Centrifuge cells at 300xg for 5 minutes
2. Remove supernatant completely and resuspend cell pellet in 100ul of Dead Cell Removal MicroBeads for every 1mil cells
3. Mix well and incubate for 15 minutes at room temperature
4. Place the MS or LS column in the magnet
5. Rinse column with 500ul (MS) or 3ml (LS) of 1X Binding Buffer
6. Apply cell suspension into the column
7. Rinse with 500ul (MS) or 1ml (LS) of 1X Binding buffer
8. Collect effluent
9. Rinse and collect with 4x500ul (MS) or 4x3ml (LS) of 1X Binding buffer

## Magnetic Labelling

*\*Keep cells on ice*

*\*Ensure cells are filtered prior to passing through the column, wet filter with buffer before use*

#### Buffer Prep

Make a 1L stock

PBS pH7.2

0.5% BSA

2mM EDTA

0.744g EDTA (MW372.2)

50ml 0.5% BSA

Fill to 1L with pH7.2 PBS

1. Determine cell counts
2. Centrifuge cell suspension at 300xg for 10 minutes. Aspirate supernatant completely
3. Resuspend cell pellet in 80ul of buffer for every 107 cells
4. Mix well and incubate for 15 minutes at 4C
5. Wash cells by adding 2ml of buffer per 107 cells and centrifuge at 300xg for 10 minutes. Aspirate supernatant completely
6. Resuspend up to 108 cells in 500ul buffer
7. Proceed to magnetic separation

## Magnetic separation

1. Place column in the magnet
2. Rinse column with 500ul (MS) or 3ml (LS) of buffer
3. Apply cell suspension onto the column
4. Collect unlabeled cells (CD45-) and wash column with 3x500ul (MS) or 3x3ml (LS) of buffer
5. Remove column from separator and place it in a 15ml falcon tube
6. Pipette 1ml (MS) or 5ml (LS) of buffer onto the column. Flush out the labelled cells (CD45+) but pushing the plunger into the column