

BP_SORT

Version: BluePrint

Reagents

Buffer 4 - PBS, 2 mM EDTA, 0.2% Human Serum Albumin – ice cold

Materials:

EasySep Negative Human Progenitor Enrichment Kit – 19057

EasySep Negative Human T Cell Enrichment Kit – 19051

EasySep Negative Human B Cell Enrichment Kit – 19054

EasySep Negative Human NK Enrichment Kit – 19055

15 ml Falcon - Greiner Bio-One Ltd - 188271

Procedure**Pre separation**

Resuspend PBMC cell pellet in ice cold Buffer 4 at 50×10^6 cells / ml

Transfer a maximum of 7 ml of cell suspension to a 15ml Falcon tube

Add 50 ul / ml of the appropriate EasySep enrichment cocktail

Mix well and incubate at room temperature for 10 minutes (15 minutes for Progenitor Kit)

Vortex the EasySep magnetic particles for 30 seconds

Add the EasySep magnetic particles at:

50 ul / ml for Progenitor and T cell kits

75 ul / ml for B cell kit

100 ul / ml for NK cell kit

Incubate at room temperature for 5 minutes (15 minutes for Progenitor Kit)

Bring the cell suspension to a total volume of 8 ml using ice cold Buffer 4

Mix well and place tube in magnet (StemCell – Purple Magnet) and set aside for the following times:

10 minutes for Progenitor kit

5 minutes for T and B cell kits

2.5 minutes for NK cell kit incubation

In one continuous motion, pour off the negative fraction into a 15 ml Falcon tube, leaving the magnet and tube inverted for 2 – 3 seconds. DO NOT shake off any drops that may remain in the tube mouth

Record total volume and take aliquot for cell count

Wash cells – 600 g, 6 minutes, acc 9, break 9

Pipette off supernatant

Staining

Resuspend cells in FC block in 15 ml falcon– 20 ul / test (1 test = 20 million cells)

Incubate in fridge for 20 minutes

Add antibody cocktail

Incubate in fridge for 30 minutes

Wash in 5 ml Buffer 4 – 600 g, 6 minutes, acc 9, break 9

Resuspend in Buffer 4 at a concentration of 20 million cells / ml

CD4 cells

CD25	APC	2A3	BD Pharmingen™	340907
CD25	APC	M-A251	BD Pharmingen™	555434
CD4	AF 700	RPA-T4	Biolegend	300526
CD45RA	P Blue	HI100	Biolegend	304123
CD127	PEcy7	eBioRDR5	E bioscience	25-1278-42
CD62L	FITC	Dreg 56	BD Pharmingen™	555543

CD8 cells

CD45RA	P Blue	HI100	Biolegend	304123
CD62L	APC	Dreg 56	BD Pharmingen™	559772
CD3	FITC	UCHT1	BD	555332
CD8	PE	RPA-T8	BD Pharmingen™	555367

B cells

CD27	PE	M-T271	BD Pharmingen™	555441
IgD	FITC	IA6-2	BD Pharmingen™	555778
CD19	APC	HIB19	BD	555415
CD38	PerCP/Cy5.5	HIT2	BD	551400

NK

CD3	FITC	UCHT1	BD	555332
CD56	APC	N-CAM	BD	555518
CD16	PE	B73.1 / leu11c	BD	332779