

Culturing PBMC For RBC Differentiation

Stemspan (StemCell 09600 or 09650)

- bovine serum albumin
- rh insulin
- human transferrin (Iron saturated)
- 2-mercaptoethanol
- L-glutamine
- Iscove's MDM

Or: Make up own (so no BME) with BITS 09500 (StemCell) 5X- add glutamine

- bovine serum albumin (pre-buffered with NaHCO_3)
- rh Insulin
- human Transferrin (iron saturated)
- Iscove's MDM

Add cytokines

	Source	Concentration	Final Concentration	Dilution	Add to 10 ml
IL-3	Cellscience CRI113-002	2 ug/ml	1 ng/ml	1:2000	5 ul
Ep0		20,000 u/ml	1 u/ml	1:20,000	0.5 ul
Scf	Cellscience CRS200A	2 ug/ml	10 ng/ml	1:200	50 ul
Dex	Greg	1 mM	1 uM	1:1000	10 ul

Culture at $1 \times 10^6/\text{ml}$ media- my PBMC do not grow much X 10 days, start to grow and differentiate at day 10

Transfer to differentiation media

Only epo and insulin (in stemspan already)

To check Differentiation of Cells

Wash 1×10^6 cells with PBS

Resuspend in 100 ul

Add 20 ul PE CD45 (eBioscience 12-0459)

Add 0.5 ul FITC glycoporphin A (eBioscience 11-9987)

30 mins in dark

Add 1 ml PBS, spin

Resuspend in 400 ul