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Fetal liver flow cytometry analysis and FACS

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We use this protocol and it's working

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Abstract

A protocol to analyze and isolate differentiation-stage-specific murine erythroid progenitors and precursors from fetal liver (E12.5) based on cell surface markers of CD71 and Ter119 using flow cytometry.

Materials

CD3e (BD Biosciences Cat# 553060, RRID:AB_394593),
CD4 (BD Biosciences Cat# 553649, RRID:AB_394969),
CD8a (BD Biosciences Cat# 553028, RRID:AB_394566),
CD45R/B220 (BD Biosciences Cat# 553086, RRID:AB_394616),
CD127 (BD Biosciences Cat# 555288, RRID:AB_395706),
CD11b (BD Biosciences Cat# 553309, RRID:AB_394773),
Ly6G/Ly6C (BD Biosciences Cat# 553125, RRID:AB_394641),
CD71-FITC (BioLegend Cat# 113806, RRID:AB_313567),
Ter119-PE (BD Biosciences Cat# 553673, RRID:AB_394986).

Set up mouse crossing and collect E12.5 fetal livers

1 Male and female heterozygous mice were set up for mating in the evening. Males were removed from the cage on the following day, which was considered E0.5 for the embryos of pregnant females. Pregnancy was assessed by monitoring the female's weight.

2 At E12.5, embryos were removed, and tail clips were used for genotyping.

3 Fetal livers were isolated using a dissecting microscope to ensure no contamination by surrounding tissues.

Flow cytometric analysis and FACS

4 For FACS analysis, fetal livers were dissociated into single cell suspensions and passed through a 100µm nylon strainer to eliminate cell clumps and debris.

5 Percentages of live cells were determined by trypan blue staining.

6 Cells were then counted and stained with a lineage cocktail (biotinylated antibodies against CD3e, CD4, CD8a, CD45R/B220, CD127, CD11b, Ly6G/Ly6C) followed by streptavidin-PE-Cy7 (Biolegend,Cat#405206),
anti-CD71-FITC, anti-Ter119-PE, and DAPI (BD, Cat#564907).

7 Samples were analyzed on LSRII and BD FACSAriaII analyzers for flow cytometric analysis and FACS sorting, respectively

Protocol references

Koulnis M, Pop R, Porpiglia E, Shearstone JR, Hidalgo D,
Socolovsky M. Identification and analysis of mouse erythroid progenitors using the CD71/TER119 flow-cytometric assay. J Vis Exp. 2011;(54). Epub 20110805.
doi: 10.3791/2809. PubMed PMID: 21847081; PubMed Central PMCID: PMCPMC3211121.