

Protocol 610_HSC staining

Materials

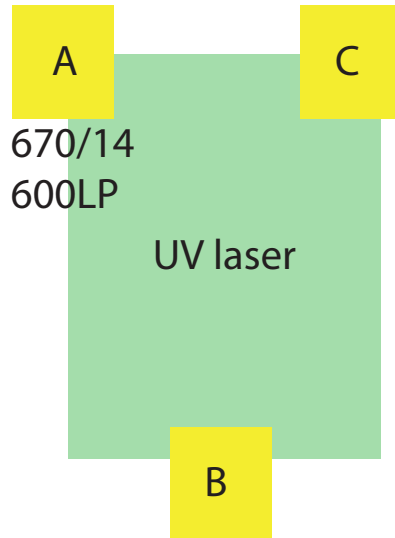
- Hoechst 33342 (Sigma Aldrich)
- HBSS+: Hanks balanced salt solution (HBSS) supplemented with 2% FCS, 1mM HEPES, 50uM penicillin/streptomycin
- DMEM+: supplemented with 10mM HEPES and 2% FCS
- Antibodies see list below

Protocol

- Prewarm DMEM medium in 37°C water bath.
- Use femurs and tibias of one mouse (both legs!) and resuspend cells in ice-cold HBSS+.
- Count cells
- Spin cells down at 1500rpm for 5min in refrigerated centrifuge
- Resuspend cell pellet at 10^6 cells/ml in prewarmed DMEM+ in 50ml Falcon tube.
- Add Hoechst (Stock concentration 1mg Hoechst in 1ml ddH₂O, stored at -20°C) to final concentration of 5µg/ml.
- Incubate cells in water bath at 37°C for exactly 90min. Mix tubes several times during incubation!
- Spin cells down in refrigerated centrifuge at 2200rpm for 5min at 4°C.
- Remove supernatant and resuspend the cells at 10^8 cells/ml in ice-cold HBSS+.
- For surface stainings take 2×10^6 cells in HBSS+
- Proceede with surface markers at correct dilution.
 - o lineage cocktail: CD11b, CD3e, B220, GR-1, NK1.1, Ter119 (all Biotin-Streptavidin-APC-Cy7)
 - o CD117 (c-kit)-APC, Sca-1 - FITC
 - o CD127(IL7-R α)-PE for CLP cells.
- Wash cells with 1-2ml HBSS+
- Store in 100µl HBSS+ and add 7-AAD (1:1000 dilution) just before acquisition. Incubate for 15min and add 200µl ice-cold HBSS+
- Cells have to be always kept on ice or in the fridge!!
- After acquisition on FACS machine export files as FCS 2.0 files (not 3.0) in order to display the Hoechst staining in linear mode in flowjoe.

Filter exchange at SORP LSRII

Normal filters in A
525/50
505LP



For detecting Hoechst red choose Hoechst
and for Hoechst blue choose Alexa 430

Voltages for BM at SORP LSRII for compensation

- Hoechst: 306 (linear mode for compensation and acquisition)
- Alexa F 430 611 (linear mode for compensation and acquisition)
- 7-AAD 625

Antibody overview

	flurophore	dilution	source	clone	Detector
Hoechst Red		5ug/ml			UV B
Hoechst Blue		5ug/ml			UV A
CD117	APC	1/100	BL	2B8	Red C
Sca-1	FITC	1/100	BD	E13-161.7	Blue D
CD127 (IL7-Ra)	PE	1/100	BD	A7R3	Blue C
7-AAD	7-AAD	1/1000			Blue B
CD11b	Biotin	1/100	BL	53-7.3	
CD3e	Biotin	1/100	BD	145-2C11	
B220	Biotin	1/100	SB	RA3-6B2	
Gr-1	Biotin	1/500	SB	RB6-8C5	
NK1.1	Biotin	1/100	BD	PK136	
Ter119 (Iy7-6)	Biotin	1/100	BD	Ter119	
Streptavidin	PE Cy7	1/200	BD		Blue A