

Materials List for:

Techniques for the Analysis of Extracellular Vesicles Using Flow Cytometry

Heather Inglis¹, Philip Norris^{1,2,3}, Ali Danesh^{1,3}

¹Blood Systems Research Institute

²Department of Medicine, University of California, San Francisco

³Department of Laboratory Medicine, University of California, San Francisco

Correspondence to: Philip Norris at pnorris@bloodsystems.org

URL: <https://www.jove.com/video/52484>

DOI: [doi:10.3791/52484](https://doi.org/10.3791/52484)

Materials

Name	Company	Catalog Number	Comments
LSR II benchtop flow cytometer	BD Biosciences		3-laser (20 mW Coherent Sapphire 488 nm blue, 25 mW Coherent Vioflame 405 nm violet, and 17 mW JDS Uniphase HeNe 633 nm red)
FACS Diva software	BD Biosciences		PC version 6.0
FlowJo software	Treestar US		Mac version 9.6.1 or PC version 7.6.5
Sphero Rainbow fluorescent particles	BD Biosciences	556298	used to adjust all channel voltages to maintain fluorescence intensity consistency
Ultra Rainbow fluorescent particles	Spherotech	URFP-10-5	used in addition to Megamix-Plus SSC beads to ensure EV gating consistency from batch to batch
Megmix-Plus SSC beads	Biocytex	7803	used to adjust FSC and SSC voltages to maintain consistency between runs. Can also used to monitor flow rate and adjust flow rate dial in order to ensure that same flow rate is used in all runs
AbC Anti-Mouse Bead Kit	Life Technologies	A-10344	used for compensation controls & negative AbC beads used for beads-based method
Nonidet P-40 Alternative (NP-40) (CAS 9016-45-9)	Santa Cruz	sc-281108	used in the individual detection method only to lyse samples after initial reading for use as negative controls. Stock may be diluted to 1:10 in PBS and stored in fridge for up to 1 month.
BD TruCOUNT Tubes	BD Biosciences	340334	used whenever absolute EV concentrations are needed
Ultrafree-MC, GV 0.22 µm Centrifugal Filter Units	Millipore	UFC30GVNB	used to post-stain wash Evs and/or fractionate EVs based on size
Vacutainer glass whole blood tubes ACD-A	BD Biosciences	364606	
Facs tubes 12x75 polystyrene	BD Biosciences	352058	
50 ml Reservoirs individually wrapped	Phenix	RR-50-1s	
Green-Pak pipet tips - 10 µl	Rainin	GP-L10S	
Green-Pak pipet tips -200 µl	Rainin	GP-L250S	
Green -Pak pipet tips - 1,000 µl	Rainin	GP-L1000S	
Stable Stack L300 tips presterilized	Rainin	SS-L300S	

Pipet-Lite XLS 8 Channel LTS Adjustable Spacer	Rainin	LA8-300XLS	
96 well tissue culture plates	E&K Scientific	EK-20180	
RPMI 1640 Media (without HEPES)	UCSF Cell Culture Facility	CCFAE001	media used for bead-based detection method
Dulbeccos PBS D-PBS, CaMg- free, 0.2 µm filtered	UCSF Cell Culture Facility	CCFAL003	
Ultracentrifuge Tube, Thinwall, Ultra-Clear	BECKMAN COULTER INC	344058	
PANEL I			
CD3 PerCP-Cy5.5	Biolegend	344808	2 µl
CD14 APC-Cy7	Biolegend	301820	2 µl
CD16 V450	BD Biosciences	560474	2 µl
CD28 FITC	biolegend	302906	2 µl
CD152 APC	BD Biosciences	555855	2 µl
CD19 A700	Biolegend	302226	2 µl
PANEL II			
CD41a PerCP-Cy5.5	BD Biosciences	340930	2 µl
CD62L APC	Biolegend	304810	2 µl
CD108 PE	BD Biosciences	552830	2 µl
CD235a FITC	biolegend	349104	2 µl
PANEL III			
CD11b PE-Cy7	Biolegend	301322	2 µl
CD62p APC	Biolegend	304910	2 µl
CD66b PE	Biolegend	305106	2 µl
CD15 FITC	exalpha	X1496M	5 µl
CD9 PE	Biolegend	555372	
CD63 APC	Biolegend	353008	
APC-Cy7 Ms IgG2a, κ	Biolegend	400230	