

Materials List for

# Preparation of Single-cell Suspension of Mouse Thymic Epithelial Cells and Staining of Intracellular Molecules for Flow Cytometric Analysis

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jove.com/video/66945

## Materials

Name	Company	Catalog Number	Comments
Collagenase/Dispase	Roche	11097113001	
Liberase TM	Roche	5401127001	
Foxp3 / Transcription Factor Staining Buffer Set	eBioscience	00-5523-00	
Ghost Dye Violet 510	Tonbo	13-0870-T500	
BV421 anti-mouse CD45 monoclonal antibody, Clone 30-F11	BD Horizon	563890	
Alexa Fluor 647 anti-mouse Ly51 monoclonal antibody, Clone 6C3	BioLegend	108312	
PE/Cy7 anti-mouse EpCAM/CD326 monoclonal antibody, Clone G8.8	BioLegend	118216	
DyLight 594 Ulex Europaeus Agglutinin I (UEA1)	Vector Laboratories	DL-1067-1	
Alexa Fluor 488 anti-mouse Aire monoclonal antibody, Clone 5H12	eBioscience	53-5934-82	
Anti-mouse Psmb11/beta5t rabbit monoclonal antibody, Clone CPTC-PSMB11(mouse)-1	NIH NCI	CPTC-PSMB11(mouse)-1	<a href="https://proteomics.cancer.gov/antibody-portal">https://proteomics.cancer.gov/antibody-portal</a>
Anti-mouse CCL21/exodus 2 rabbit polyclonal antibody	Bio-Rad	AAM27	
Alexa Fluor 555 anti-rabbit IgG heavy chain goat recombinant antibody	Invitrogen	A27039	
Anti-Mouse CD32/CD16 – Purified (Fc Block Antibody)	Leinco Technologies	C247	
Nylon Mesh 60 Micron	Component Supply	W31436	
Equipment			
Thermomixer	Eppendorf	Heat block	

Cellometer Auto 2000 Cell Viability counter	Nexcelom	Viable cell counting	
LSRFortessa Cell Analyzer	BD Biosciences	Flow cytometer	Five lasers (355, 408, 488, 595, and 637 nm)
FACSDiva software version 9.0.1	BD Biosciences	Flow cytometric analysis	
FlowJo 10.9.0	BD Biosciences	Flow cytometric data analysis	
Antibody	Dilution rate (Working concentration)	Dilution Buffer	
Anti-Mouse CD32/CD16 – Purified (Fc Block Antibody)	1:40	FACS Buffer	
BV421 anti-mouse CD45 monoclonal antibody	1:25	FACS Buffer	
PE/Cy7 anti-mouse EpCAM/CD326 monoclonal antibody	1:100	FACS Buffer	
Alexa Fluor 647 anti-mouse Ly51 monoclonal antibody	1:100	FACS Buffer	
DyLight 594 Ulex Europaeus Agglutinin I (UEA1)	1:400	FACS Buffer	
Anti-mouse CCL21/exodus 2 rabbit polyclonal antibody	1:200	1x Permeabilization Buffer	
Anti-mouse Psmb11/beta5t rabbit monoclonal antibody, Clone CPTC-PSMB11(mouse)-1	1:1000	1x Permeabilization Buffer	
Alexa Fluor 555 anti-rabbit IgG heavy chain goat recombinant antibody	1:400	1x Permeabilization Buffer	
Buffer	Purpose		
RPMI1640 containing 10 mM HEPES	For cell preparation		
RPMI1640 containing 2% FBS	For cell preparation		
FACS buffer (1x HBSS containing 1% BSA and 0.1% sodium azide)	For cell preparation and antibody staining		
1 mg/ml Collagenase/Dispase and 1U/ml DNase solution in RPMI1640 containing 2% FBS	For enzyme digestion		
2% PFA in PBS	For cell fixation		
Fixation/Permeabilization Buffer (1 part of Fixation/Permeabilization Concentrate with 3 parts of Fixation/Permeabilization Diluent)	For cell fixation		
1x Permeabilization Buffer (1 part 10X concentrate with 9 parts distilled water)	For intercellular antibody staining		