

Materials List for:

Human Lung Dendritic Cells: Spatial Distribution and Phenotypic Identification in Endobronchial Biopsies Using Immunohistochemistry and Flow Cytometry

Faezzah Baharom¹, Gregory Rankin², Saskia Scholz¹, Jamshid Pourazar², Clas Ahlm³, Anders Blomberg², Anna Smed-Sörensen¹

¹Immunology and Allergy Unit, Department of Medicine Solna, Karolinska Institutet

²Division of Medicine, Department of Public Health and Clinical Medicine, Umeå University

³Division of Infectious Diseases, Department of Clinical Microbiology, Umeå University

Correspondence to: Anna Smed-Sörensen at Anna.Smed.Sorensen@ki.se

URL: <https://www.jove.com/video/55222>

DOI: [doi:10.3791/55222](https://doi.org/10.3791/55222)

Materials

Name	Company	Catalog Number	Comments
Bronchoscopy			
Bronchoscope BF1T160	Olympus	BF1T160	
Light source	Olympus	Exera CV-160	
Fenestrated forceps	Olympus	FB21C	Used to take biopsies
Bite Block	Conmed	1429	20 mm x 27 mm
Glucose 25%			500 ml intravenous
Glycopyrronium bromide 0.2 mg/ml			Intravenous. Prevents mucus/saliva secretion
Mixt. Midazolam 1 mg/ml p.o			Can be used for extra relaxation
Lidocaine, 40 mg/ml			Mouth and throat administration / Gargled
Lidocaine 100 mg/ml spray			Administered to back of throat
Lidocaine 20 mg/ml spray			Administered via bronchoscope to airways
GMA processing and embedding			
Glass vials			5 ml
Acetone	Sigma-Aldrich	32201-1L	
Molecular sieves, 4 Å	Alfa Aesar	88120	3-4 mm diameter pellets
Phenylmethylsulfonyl fluoride	Sigma-Aldrich	P-7626	0.035 g/100 ml acetone
Iodoacetamide	Sigma-Aldrich	I-6125	0.37 g/100 ml acetone
Polythene-flat TAAB embedding capsules	TAAB laboratories	C094	500x 8 mm diameter, polythene, flat-bottom capsules
Capsule holder	TAAB laboratories	C054	Holds 25 8 mm capsules
JB-4 GMA embedding kit	Polysciences	00226	Contains JB-4 Solution A (0026A-800), JB-4 solution B (0026B-3.8), benzoyl peroxide (02618-12)
Methyl benzoate	Sigma-Aldrich	27614-1L	
Silica gel with humidity indicator	Scharlau	GE0043	2.5-6 mm
GMA sectioning			
Glass microscope slides	ThermoFisher Scientific	10143562CEF	Cut edges, frosted end
Poly-L-Lysine solution	Sigma-Aldrich	P8920-500mL	1:10 for working solution
Sheet glass strips for ultramicrotomy	Alkar		

Tween 20	Sigma-Aldrich	P2287	Wash solution (0.1% Tween20)
LKB 7800B Knifemaker	LKB		
Capsule splitter	TAAB laboratories	C065	
Carbon steel single edge blades	TAAB laboratories	B054	
Vice			
Ammonia, 25%	VWR	1133.1000	2 ml in 1 L, 1:500 (0.05%)
Microtome	Leica		Leica RM 2165
Light source	Leica		Leica CLS 150 XE
Microscope with swing arm stand	Leica		Leica MZ6
GMA Immunohistochemistry			
Diamond tipped pen	Histolab	5218	
Hydrogen peroxide 30% solution	AnalaR Normapur	23619.264	
Sodium azide	Sigma-Aldrich	S8032	
Tris	Roche	10708976001	
Sodium chloride	VWR chemicals	27810.295	
Bovine serum albumin	Millipore	82-045-2	Probumin BSA diagnostic grade
Dulbecco's modified eagle medium (DMEM)	Sigma-Aldrich	D5546	
Anti-human CD45 antibody	BioLegend	304002	Mouse monoclonal, clone HI30, isotype IgG1k. Working concentration of 500 ng/ml
Anti-human CD1a antibody	AbD Serotech	MCA80GA	Mouse monoclonal, clone NA1/34-HLK, isotype IgG2a. Working concentration of 10 µg/ml
Mouse monoclonal IgG1 isotype control	Abcam	ab27479	
Mouse monoclonal IgG2a isotype control	Dako	X094301-2	
Vectastain ABC Elite standard kit	Vector Labs	PK-6100	
AEC (3-amino-9-ethylcarbazole) peroxidase substrate kite	Vector Labs	SK-4200	
Mayers haematoxylin	HistoLab	01820	
Permanent Aqueous Mounting Medium	AbD Serotech	BUF058C	
Drying oven			
DPX permanent mounting solution	VWR	360292F	
Light microscope	Leica		Leica DMLB
Microscope camera	Leica		Leica DFC 320
Analysis software	Leica		Leica Qwin V3
Enzymatic digestion			
Hank's Balanced Salt Solution (HBSS)	Sigma-Aldrich	55021C	
Dithiothreitol (DTT)	Sigma-Aldrich	DTT-RO	
Collagenase II	Sigma-Aldrich	C6885	
DNase	Sigma-Aldrich	10104159001 ROCHE	
RPMI 1640	Sigma-Aldrich	R8758	
Forceps			
Platform rocker	Grant instruments	PMR-30	
50 ml conical tubes	Falcon	14-432-22	

40 µm cell strainer	Falcon	352340	
Flow cytometry			
Phosphate Buffered Saline (PBS)			
LIVE/DEAD Aqua fixable dead cell stain kit	Life Technologies	L34957	
CD45	BD	555485	
CD3	BD	557757	
CD20	BD	335829	
CD56	Biolegend	318332	
CD66abce	Miltenyi	130-101-132	
HLA-DR	BD	555813	
CD14	BD	557831	
CD16	Biolegend	302026	
CD11c	BD	560369	
CD1c	Miltenyi	130-098-009	
CD141	Miltenyi	130-090-514	
CD103	Biolegend	350212	
Paraformaldehyde	Sigma-Aldrich	F8775	
LSR II Flow cytometer	BD		Flow cytometer
FlowJo	FlowJo		Software for analysis