

Materials List for:

Automated Quantification of Hematopoietic Cell – Stromal Cell Interactions in Histological Images of Undecalcified Bone

Sandra Zehentmeier¹, Zoltan Cseresnyes^{2,3}, Juan Escribano Navarro⁴, Raluca A. Niesner², Anja E. Hauser^{1,5}

¹Immunodynamics, German Rheumatism Research Center, a Leibniz Institute

²Biophysical Analytics, German Rheumatism Research Center, a Leibniz Institute

³Max-Delbrück Center for Molecular Medicine

⁴Wimasis GmbH















⁵Immunodynamics and Intravital Imaging, Charité - University of Medicine







Correspondence to: Anja E. Hauser at hauser@drfz.de

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Materials

Name	Company	Catalog Number	Comments
Neomycin	 Sigma	N6386 SIGMA	Neomycin trisulfate salt hydrate, EU hazard code: GHS08
Ursovit AD3EC	Serumwerke Bernburg		1 ml contains: 50.000 I.E. retinyl palmitate, 5.000 I.E. cholecalciferol, 30 mg tocopheryl acetate, 100 mg ascorbic acid, 1 mg sorbic acid, 200 mg polyoxyl 35 castor oil, 0,5 mg propyl gallate
Transfer buffer (100 ml PBS, 1 ml 1 M HEPES, 50 U/ml penicillin/streptomycin)	 Sigma	P4333, H3375	
4-Hydroxy-3-nitrophenylacetyl hapten conjugated to chicken gamma globulin			
Chicken gamma globulin (CGG) 100 mg	 Rockland	D602-0100	
20% Paraformaldehyde solution (EM-grade)	Science Services	15713	EU hazard codes: GHS02, GHS05, GHS07, GHS08
D(+)-sucrose	 Carl Roth	4621.1	
Dry ice			
Acetone	 Sigma-Aldrich	179124 SIGMA-ALDRICH	EU hazard codes: GHS02, GHS07
Hexane	 Sigma-Aldrich	208752 SIGMA-ALDRICH	EU hazard codes: GHS02, GHS07, GHS08, GHS09
Tissue-Tek cryomolds (standard)	 Sakura	4557	25 x 20 x 5 mm
Tissue-Tek O.C.T.	 Sakura	4583	
Kawamoto's SCEM embedding medium	 Section-Lab, JP		
Kawamoto's cryosection preparation kit	 Section-Lab, JP		
Kawamoto's cryofilm type 2C(9)	 Section-Lab, JP		
Microtome blade MX35 premier plus, low profile	 Thermo Scientific	3052835	L X W: 80 x 8 mm (31.5 x 3.13"), thickness: 0.25 mm (0.01")
Polyclonal rabbit anti-RFP antibody, biotinylated	 Rockland	600-406-379	
Alexa Fluor 555 streptavidin	 Life Technologies	S-32355	

Rat anti-MBP	J. and N. Lee		available from: J. and N. Lee, Mayo Clinic, Scottsdale, AZ , U.S.A., clone MT-14.7
Goat anti-rat-Alexa Fluor 647	 Life Technologies	A-21247	
Rat anti-B220 - Alexa Fluor 594			produced and coupled in-house (DRFZ), clone RA3.6B2, Alexa Fluor 594 from Life Technologies
Mouse anti-I1 light chain -FITC			produced and coupled in-house (DRFZ), clone LS136
Rat anti-k light chain - FITC			produced and coupled in-house (DRFZ), clone 187.1
DAPI (4',6-diamidino-2-phenylindole dihydrochloride)	 Sigma	D9542 SIGMA	
Fluorescent mounting medium	 DAKO	S3023	
Cover slips (24 x 24 x 0.13-0.16 mm)	 Carl Roth	H875.2	
Superfrost slides glasses (75 x 25 mm)	 VWR	48311-703	
Laser scanning confocal microscope			equipped with laser lines of 405, 488, 561, 594, 633 nm and a 20X/0.8 NA air objective lens. We used a Zeiss LSM710 and Zen 2010 Version 6.0 software.
Automated image analysis tools for bone marrow	 Wimasis, Munich		The cell contact tool and cell vicinity tool will be made available by Wimasis upon request.
VC2012 runtime	Microsoft		free download
Simulation tool for random cell positioning			available from us, upon request
Image analysis software with image segmentation functions			We used Volocity (Perkin Elmer) for measuring cell size distributions of hematopoietic cell types in bone marrow (Figure 5). Alternatives are Definiens Image Analysis (Definiens) or Cell Profiler (free download)
Fiji image analysis software			free download . Fiji was used by trained raters for manual cell count (Figure 3).