

Materials List for:

Visualizing the Actin and Microtubule Cytoskeletons at the B-cell Immune Synapse Using Stimulated Emission Depletion (STED) Microscopy

Jia C. Wang¹, Madison Bolger-Munro¹, Michael R. Gold¹

¹Department of Microbiology and Immunology, University of British Columbia

Correspondence to: Michael R. Gold at mgold@mail.ubc.ca

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Materials

Name	Company	Catalog Number	Comments
Cell culture			
A20 mouse B-lymphoma cells	ATCC	TIB-208	Murine B-cell lymphoma of Balb/c origin that expresses an IgG-containing BCR on its surface
RPML-1640	Thermo Fisher Scientific	R0883	
Fetal bovine serum	Thermo Fisher Scientific	12483020	Heat inactivate at 56 °C for 30 min
2-mercaptoethanol	Millipore Sigma	M3148	
Glutamine	Millipore Sigma	G5763	
Sodium pyruvate	Millipore Sigma	P5280	
Penicillin-Streptomycin	Thermo Fisher Scientific	15140122	Liquid, 10,000 units
Additional materials for primary B cells			
70-µm cell strainer	Corning	352350	
Magnetic bead-based B cell isolation kit	Stemcell Technologies	19854	EasySep Mouse B cell Isolation kit
Lipopolysaccharide (LPS)	Millipore Sigma	L4391	LPS from <i>E. coli</i> 0111:B4
B cell-activating factor (BAFF)	R&D Systems	2106-BF-010	
Transfection			
Plasmid encoding CLIP-170-GFP			Gift from Dr. Kozo Kaibuchi (Nagoya Univ., Nagoya, Japan); described in Fukata <i>et al. Cell</i> 109:873-885, 2002
Recommended transfection reagents and equipment			
Amaya Nucleofection kit V	Lonza	VCA-1003	Follow the manufacturer's directions for mixing the transfection reagents with the DNA
Amaya Nucleofector model 2b	Lonza	AAB-1001	Program L-013 used
Falcon 6-well plates, TC treated, sterile	Corning	353046	
Coating coverslips			
18-mm diameter round #1.5 cover glasses	Thomas Scientific	1217N81	Similar product: Marienfeld-Superior catalogue #117580
Forceps	Fisher Scientific	1381242	Dissecting extra fine splinter forceps

Falcon 12-well sterile tissue polystyrene tissue culture plate	Corning	353043	
100% methanol	Fisher Scientific	A412-4	
Sterile phosphate buffered saline (PBS) without calcium or magnesium	Thermo Fisher Scientific	10010049	
Goat-anti-mouse IgG antibody	Jackson ImmunoResearch	115-005-008	For A20 cells
Goat-anti-mouse IgM antibody	Jackson ImmunoResearch	115-005-020	For primary mouse B cells
Name	Company	Catalog Number	Comments
Staining			
Paraformaldehyde (16% stock solution)	Electron Microscopy Sciences	15710	Dilute with PBS to working concentration
Glutaraldehyde (50% stock solution)	Millipore Sigma	340855	Dilute with PBS to working concentration
Triton X-100	Fisher Scientific	BP151-500	
Saponin	Millipore Sigma	S2149	
Bovine serum albumin (BSA), Fraction V	Millipore Sigma	10735094001	
Rabbit anti-tubulin antibody	Abcam	ab52866	1:250 dilution recommended but should be optimized
Alexa Fluor 532-conjugated goat anti-rabbit IgG	Thermo Fisher Scientific	A11009	1:100 dilution recommended but should be optimized
Alexa Fluor 568-conjugated phalloidin	Thermo Fisher Scientific	A12380	1:100 dilution recommended but should be optimized
Prolong Diamond Antifade Mountant	Thermo Fisher Scientific	P36970	Without DAPI
Fisherbrand Superfrost Plus Microscope Slides	Fisher Scientific	12-550-15	
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Materials			
Parafilm	VWR	P1150-2	
HEPES	Millipore Sigma	H3375	
NaCl	Fisher Scientific	BP358	
KCl	Millipore Sigma	P9333	
CaCl ₂	Millipore Sigma	C1016	
Na ₂ HPO ₄	Fisher Scientific	S374-500	
MgSO ₄	Fisher Scientific	M63	
Dextrose	Fisher Scientific	D16-500	
Name	Company	Catalog Number	Comments
Microscopy			
Leica SP8 TCS STED microscope	Leica		
Huygens deconvolution software	Scientific Volume Imaging		See https://svi.nl/HuygensProducts