

Materials List for:

Characterizing the Composition of Molecular Motors on Moving Axonal Cargo Using "Cargo Mapping" Analysis

Sylvia Neumann¹, George E. Campbell^{*1}, Lukasz Szpankowski^{*2,3}, Lawrence S.B. Goldstein^{2,4}, Sandra E. Encalada¹

¹Department of Molecular and Experimental Medicine, Dorris Neuroscience Center, The Scripps Research Institute

²Department of Cellular and Molecular Medicine, University of California San Diego

³Department of Bioengineering, University of California San Diego

⁴Department of Neurosciences, University of California San Diego School of Medicine

* These authors contributed equally

Correspondence to: Sandra E. Encalada at encalada@scripps.edu

URL: <https://www.jove.com/video/52029>

DOI: [doi:10.3791/52029](https://doi.org/10.3791/52029)

Materials

Name	Company	Catalog Number	Comments
poly-L-lysine	Sigma	P5899-20mg	
D-MEM (Dulbecco's Modified Eagle Medium) High Glucose, w/ L-Glutamine, w/o Sodium Pyruvate (1x)	Life Technologies	11965092	
FBS (Fetal Bovine Serum)	Life Technologies	10082147	
Neurobasal-A Medium (1x)	Life Technologies	10888022	
B-27 Serum Free Supplement	Life Technologies	10888022	
GlutaMAX I Supplement (100x)	Life Technologies	35050061	
HBSS (1x) (Hank's Balanced Salt Solution)	Life Technologies	24020117	
DPBS (Dulbecco's Phosphate Buffered Saline, no Magnesium, no Calcium (1x)	Life Technologies	14190250	
Penicillin/Streptomycin (100x)	Life Technologies	15140122	
Corning cellgro Water for Cell Culture	Fisher Scientific	MT46000CM	
Papain	USB Corporation	19925	
DL-cysteine HCl	Sigma-Aldrich	C9768	
BSA (bovine serum albumin)	Sigma-Aldrich	A7906	
D-glucose	Sigma-Aldrich	G6152	
DNAse I grade II	Roche Applied Sciences	10104159001	
Lipofectamine 2000	Life Technologies	11668027	
Formaldehyde Solution 16% EM Grade	Fisher Scientific	50980487	Caution: Harmful by inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system and skin. Dispose according to official regulations.
Sucrose	Fisher Scientific	S5-500	
HEPES	Sigma	H-3375	
Normal Donkey Serum	Jackson Immuno Research	017-000-0121	
BSA fatty acid and IgG free	Jackson Immuno Research	001-000-162	
Acetone	Fisher Scientific	BP2403-4	
Ethanol	Fisher Scientific	BP2818-100	

ProLong Gold antifade reagent	Life Technologies	P36934	
Cover Glass 1 1/2. 24 x 40 mm	Corning	2980-244	
Axis microfluidic device, 450 µm	Millipore	AX450	
Adobe Photoshop	Adobe		
Nikon Eclipse TE2000-U	Nikon		
Coolsnap HQ camera	Roper Scientific		
60 mm cell culture dish	Fisher Scientific	12-565-95	
150 mm cell culture dish	Fisher Scientific	12-565-100	
Antibodies Used:			
Anti-Kinesin light chain, V-17	Santa Cruz	sc-13362	specificity verified using KLC1-/- neurons (Ref. 16), recommended dilution 1:100.
Anti Dynein Heavy Chain 1, R-325	Santa Cruz	sc-9115	specificity verified using shRNA against DYN1HC1 (Ref. 16), recommended dilution 1:100
Alexa Fluor 568 Donkey Anti-Rabbit IgG Antibody	Life Technologies	A10042	recommended dilution 1:200.
Alexa Fluor 647 Donkey Anti-Rabbit IgG (H+L) Antibody	Life Technologies	A31573	recommended dilution 1:200.
Alexa Fluor 568 Donkey Anti-Goat IgG (H+L) Antibody	Life Technologies	A11057	recommended dilution 1:200.
Alexa Fluor 647 Donkey Anti-Goat IgG (H+L) Antibody	Life Technologies	A21447	recommended dilution 1:200.
Plugins and Macros			
ImageJ			http://imagej.nih.gov/ij/index.html .
ImageJ Kymograph Plugin			http://www.embl.de/eamnet/html/body_kymograph.html .