

Reconstituting and Characterizing Actin-Microtubule Composites with Tunable Motor-Driven Dynamics and Mechanics

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Materials

Name	Company	Catalog Number	Comments
(-)-Blebbistatin Abbreviation used in paper: blebbistatin	Sigma Aldrich	B0560	Stock Concentration: 200 μ M in DMSO Storage: dessicated, in DMSO, -20°C Stock and Experiment Recipes: dissolve 1 mg of powder to 200 μ M in DMSO Storage, Handling, Troubleshooting Notes: limited shelf-life, typically stops functioning reliably after 3-4 months. purchase and prepare new solution every 3 months.
1:20 488-tubulin:tubulin mixture Abbreviation used in paper: 5-488-tubulin	NA	NA	Stock Concentration: 5 mg/ml in PEM Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: mix tubulin and 488-tubulin at a 20:1 ratio, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: each aliquot can be used for up to 12 hrs stored on ice at 4°C, protect from light
1:20 R-tubulin:tubulin mixture Abbreviation used in paper: 5-R-tubulin	NA	NA	Stock Concentration: 5 mg/ml in PEM Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: mix tubulin and rhodamine tubulin at a 20:1 ratio, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: each

			aliquot can be used for up to 12 hrs stored on ice at 4°C, protect from light
actin (biotin): skeletal muscle Abbreviation used in paper: biotin-actin	Cytoskeleton	AB07	Stock Concentration: 1 mg/ml in G-buffer Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: reconstitute to 1 mg/ml in G-buffer, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: (1) immediately prior to use dilute to 0.5 mg/ml in PEM, (2) once removed from -80°C, store aliquot on ice at 4°C for up to 1 week
actin (rhodamine): rabbit skeletal muscle Abbreviation used in paper: R-actin	Cytoskeleton	AR05	Stock Concentration: 1.5 mg/ml in G-buffer Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: reconstitute to 1.5 mg/ml in G-buffer, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: once removed from -80°C, store aliquot on ice at 4°C, can be used for up to 1 week
adenosine triphosphate Abbreviation used in paper: ATP	Thermo Fisher Scientific	A1048	Stock Concentration: 100 mM Storage: in solution (pH 7), -20°C Stock and Experiment Recipes: reconstitute in DI H ₂ O, bring pH to 7 with NaOH Storage, Handling, Troubleshooting Notes: routinely check pH and adjust as needed, hydrolyzes over time, replace every ~6-12 months
AlexaFluor488 Phalloidin Abbreviation used in paper: 488-phalloidin	Thermo Fisher Scientific	A12379	Stock Concentration: 100 µM DMSO Storage: protected from light, desiccated, -20°C Stock and Experiment Recipes: reconstitute to 100 µM with DMSO Storage, Handling, Troubleshooting Notes: immediately prior to use dilute to 20 µM in PEM (1 µL in 4 µL PEM)
AlexaFluor488-labeled actin Abbreviation used in paper: 488-actin	Thermo Fisher Scientific	A12373	Stock Concentration: 1.5 mg/ml in G-buffer Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: reconstitute to 1.5 mg/ml in G-buffer, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: this item has been discontinued
Basic Plasma Cleaner Abbreviation used in paper: plasma cleaner	Harrick Plasma	PDC-32G	
Bemis Parafilm M Laboratory Wrapping Film Abbreviation used in paper: transparent film	Thermo Fisher Scientific	13-374-5	
D-(+)-Glucose Abbreviation used in paper:	Thermo Fisher Scientific	A1682836	Stock Concentration: 100x

			<p>Storage: store at stock concentration (100x) or 10x concentration, desiccated, at -20°C</p> <p>Stock and Experiment Recipes: reconstitute powder to 4.5 mg/ml in DI H₂O</p> <p>Storage, Handling, Troubleshooting Notes: final concentration in solution should 45 µg/mL</p>
D-Biotin Abbreviation used in paper: biotin	Fisher Scientific	BP232-1	<p>Stock Concentration: 1.02 mM in PEM</p> <p>Storage: desiccated, 4°C</p>
deionized nanopure water Abbreviation used in paper: DI			
Dimethyldichlorosilane Abbreviation used in paper: silane	Thermo Fisher Scientific	D/3820/PB05	Stock Concentration: 2% dissolved in Toulene
Dithiothreitol Abbreviation used in paper: DTT	Thermo Fisher Scientific	R0861	<p>Stock Concentration: 1 M in DMSO</p> <p>Storage: desiccated, -20°C</p> <p>Stock and Experiment Recipes: dilute to 2 mM in PEM immediately before each experiment</p>
DMSO Anhydrous Abbreviation used in paper: DMSO	Thermo Fisher Scientific	D12345	
F-Buffer Abbreviation used in paper: F-buffer	NA	NA	<p>Stock Concentration: 10x</p> <p>Storage: desiccated, -20°C</p> <p>Stock and Experiment Recipes: 10 mM Imidazole (pH 7.0), 50 mM KCl, 1 mM MgCl₂, 1 mM EGTA, 0.2 mM ATP</p>
G-Buffer Abbreviation used in paper: G-buffer	NA	NA	<p>Stock Concentration: 10x</p> <p>Storage: desiccated, -20°C</p> <p>Stock and Experiment Recipes: 2.0 mM Tris (pH 8), 0.2 mM ATP, 0.5 mM DTT, 0.1 mM CaCl₂. Store at -20°C.</p>
glass microscope slide Abbreviation used in paper: slide	Thermo Fisher Scientific	22-310397	
Glucose oxidase + catalase + β-mercaptoethanol Abbreviation used in paper: GOC	Sigma Aldrich	G2133-250KU, C1345, 63689	<p>Stock Concentration: 100x</p> <p>Storage: store at stock concentration (100x) or 10x concentration, desiccated, at -20°C</p> <p>Stock and Experiment Recipes: For 100x: 4.3 mg/ml glucose oxidase, 0.7 mg/ml catalase, 0.5% v/v β-mercaptoethanol in DI H₂O</p> <p>Storage, Handling, Troubleshooting Notes: final concentration in solution should be: 0.005% β-mercaptoethanol, 43 µg/mL glucose oxidase, 7 µg/mL catalase</p>
glu-GOC oxygen scavenging system Abbreviation used in paper: glu-GOC	NA	NA	<p>Stock Concentration: 100x</p> <p>Storage: prepare fresh each time</p> <p>Stock and Experiment Recipes: mix equal parts Glu and GOC and add at 1/100 final sample volume immediately before imaging</p> <p>Storage, Handling, Troubleshooting Notes: prepare from Glu and GOC immediately before imaging</p>
Guanosine triphosphate Abbreviation used in paper: GTP	Thermo Fisher Scientific	R0461	<p>Stock Concentration: 100 mM</p> <p>Storage: 100 µL aliquots at -20°C</p>
Instant Mix 1-minute epoxy	Loctite	1366072	

Abbreviation used in paper: epoxy			
Kinesin-1 401 BIO 6x HIS Abbreviation used in paper: kinesin	Prepared in JL Ross Lab at Syracuse University	NA	Stock Concentration: 8.87 μ M in PEM Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Storage, Handling, Troubleshooting Notes: biotinylated dimers form kinesin clusters, each aliquot can be used for up to 12 hrs stored on ice at 4°C
NeutrAvidin Abbreviation used in paper: NA	Thermo Fisher Scientific	31000	Stock Concentration: 5 mg/ml in PEM Storage: dessicated, -20°C Stock and Experiment Recipes: reconstitute powder to 5 mg/ml in PEM
No 1. glass coverslips (24 mm x 24 mm) Abbreviation used in paper: coverslip	Thermo Fisher Scientific	12-548-CP	
Paclitaxel Abbreviation used in paper: Taxol	Thermo Fisher Scientific	P3456	Stock Concentration: 2 mM in DMSO Storage: protected from light, dessicated, -20°C Stock and Experiment Recipes: reconstitute to 2 mM with DMSO Storage, Handling, Troubleshooting Notes: immediately prior to use dilute to 200 μ M in DMSO (0.4 μ L in 3.6 μ L DMSO)
PEM-100 Abbreviation used in paper: PEM	NA	NA	Stock Concentration: 1x Storage: room temperature (RT) Stock and Experiment Recipes: 100 mM K-PIPES (pH 6.8), 2 mM EGTA, 2 mM MgCl ₂ Storage, Handling, Troubleshooting Notes: use KOH to adjust pH to 6.8, recheck pH often and adjust accordingly
phalloidin Abbreviation used in paper: phalloidin	Thermo Fisher Scientific	P3457	Stock Concentration: 100 μ M in DMSO Storage: protected from light, dessicated, -20°C, adhere closely to storage/handling conditions Stock and Experiment Recipes: reconstitute to 100 μ M with DMSO Storage, Handling, Troubleshooting Notes: susceptible to impurities in its preparation and denaturing, identifiable as large amorphous aggregates of actin in samples
porcine brain tubulin Abbreviation used in paper: tubulin	Cytoskeleton	T240	Stock Concentration: 5 mg/ml in PEM Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: reconstitute powder to 5 mg/ml in PEM, flash freeze with LN ₂ Storage, Handling, Troubleshooting Notes: each aliquot can be used for up to 12 hrs stored on ice at 4°C
Potassium Chloride Abbreviation used in paper: KCl	Thermo Fisher Scientific	AM9640G	Stock Concentration: 4 M Storage: RT

Rabbit skeletal actin Abbreviation used in paper: actin	Cytoskeleton	AKL99	Stock Concentration: 2 mg/ml in G-buffer Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: reconstitute to 2 mg/ml in G-buffer, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: once removed from -80°C, store aliquot on ice at 4°C, can be used for up to 1 week
Rabbit skeletal myosin II Abbreviation used in paper: myosin	Cytoskeleton	MY02	Stock Concentration: 10 mg/ml in PEM Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: reconstitute powder to 10 mg/ml in PEM, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: monomers form minifilaments at low KCl, each aliquot can be used for up to 12 hrs stored on ice at 4°C
Tubulin (biotin): porcine brain Abbreviation used in paper: biotin-tubulin	Cytoskeleton	T333P	Stock Concentration: 5 mg/ml in PEM Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: reconstitute powder to 5 mg/ml in PEM, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: immediately prior to use dilute to 0.5 mg/ml in PEM
Tubulin (fluorescent HiLyte 488): porcine brain Abbreviation used in paper: 488-tubulin	Cytoskeleton	TL488M	Stock Concentration: 5 mg/ml in PEM Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: reconstitute powder to 5 mg/ml in PEM, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: each aliquot can be used for up to 12 hrs stored on ice at 4°C, protect from light
tubulin (rhodamine): porcine brain Abbreviation used in paper: R-tubulin	Cytoskeleton	TL590M	Stock Concentration: 5 mg/ml in PEM Storage: single use aliquots, -80°C, avoid freeze-thaw cycles Stock and Experiment Recipes: reconstitute powder to 5 mg/ml in PEM, flash freeze with LN2 Storage, Handling, Troubleshooting Notes: each aliquot can be used for up to 12 hrs stored on ice at 4°C, protect from light
Tween 20 Abbreviation used in paper: Tween20	Thermo Fisher Scientific	J20605.AP	Stock Concentration: 1% v/v in DI H ₂ O Storage: RT
ultracentrifuge grade microtubes Abbreviation used in paper: Beckman-Coulter Optima Max XP	Beckman Coulter	343776	Storage, Handling, Troubleshooting Notes: 8x34 mm PC
UV light curing glue	Pharda	SKG-2869	

Abbreviation used in paper: UV glue			
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