jove

Materials List for

Self-Assembly of Microtubule Tactoids

Prashali Chauhan[1], Sumon Sahu[1,2], Niaz Goodbee[1], Sophia Martin[1], Hong Beom Lee[1], Ruell Branch[1], Jennifer M. Schwarz[1], Jennifer L. Ross[1]

[1]Physics Department, Syracuse University [2]Department of Molecular Biophysics and Biochemistry, Yale School of Medicine

Corresponding Author

Jennifer L. Ross
jlross@syr.edu

Date Published

June 23, 2022

URL

jove.com/video/63952

Materials

Name	Company	Catalog Number	Comments
2% Dichlorodimethylsilane	GE Healthcare	118945	A hydrophobic silane surface treatment. This can be resued upto three times and kept at room temperature for one year.
5-minute epoxy	Bob Smith Industries		For sealing experimental chambers
Acetone	Fisher	32900HPLC	100%
BL21 cells	Bio Labs	C2527I	Competent bacterial cells used to express MAP65
Catalase	Sigma	C30-500MG	Part of the oxygen scavenging system. A 300 mg/ml stock is made in ddH2O and stored in 10 µl aliquots in -20°C for upto one year.
Coverslips (22 mm x 22 mm or 22 mm x 30 mm)	Fisher	12544-AP	For experimental chambers
Dithiothreitol	Sigma	43815-5G	A small molecule that is used to break disulfide bonds and scavenge oxygen. A 1 M stock is made from powder (Sigma) in ddH2O. The solution is aliquoted and stored at -20C for upto one year. The aliquots are used 7-8 times then discarded.
EGTA	Sigma	E3889-10G	Tubulin buffer base ingredient
Eppendorf 0.5 ml tubes	Eppendorf	05 402 18	For holding experimental solutions
Ethanol	Fisher	111000200	200 proof
Filter paper	Whatman	1004-110	For cleaning and for pulling solutions through experimental chambers
Fluorescence microscope with high NA objectives	Nikon	Ti-E, W2 Confocal	For imaging experiments
Freezer -20°C	Fisherbrand	13986148	For storing reagents
Freezer -80°C	Thermo scientific	328223H01-C	For storing proteins.
Glass containers for silanization	Michaels		For preparing experimental chambers - treating cover glasses
Glass slides	Fisher	12544-4	For experimental chambers
Glucose	Sigma	G7528-250G	Part of the oxygen scavenging system. A 300 mg/ml stock is made

			in ddH2O and stored in 10 µl aliquots in -20°C.
Glucose oxidase	Sigma	G2133-250KU	Part of the oxygen scavenging system. This is stored at 4°C for upto one year.
GMPCPP	Jenna Bioscience	NU-4055	Slowly hydrolyzable analog of GTP used to polymerize and stabilize the microtubules by reducing the dynamic instability and spontaneous critical concentration for microtubule nucleation to get a consistent length. We purchase a 10 mM stock from Jena Biosciences and store it at -20°C for upto one year.
Heating element for microscope	Okolab stage top incubator		For imaging experiments
Imidizole	Sigma	I2399	Used to elute MAP65 protein from Nickel beads to purify MAP65
Kimwipes	Fisher	34155	For cleaning and for pulling solutions through experimental chambers.
KOH	Sigma	P250-500	1 M in ddH2O, made fresh to prevent acidification over time.
Liquid Nitrogen	Airgas	NI 230LT22	To drop freeze aliquots. This can also be used for storage (not recommended)
MAP65 protein	Ram Dixit		A microtubule-associated protein (MAP) with a molecular weight of 65 kD that is an antiparallel microtubule crosslinker. We have purified an unlabeled and GFP-labeled version of MAP65-1 from Arabidopsis thaliana. We mix the GFP-MAP65 with the unlabeled MAP65 such that 10% of the protein is labeled. The working stock is a 10.8 µM solution. This working solution is stored at 4°C and remade fresh every week. The protocol for purifying the MAP65 and GFP-MAP65 is given in our prior methods chapter (26).
MgSO4	Sigma	MKCJ940	Tubulin buffer ingredient
NTA-Nickel beads	Qiagen	30210	Beads required to purify 6xHis tagged MAP65 proteins
Optomicroscan	Nikon		405 nm laser system which can focus the laser in any desired shape of region of interest
PEM80			Neutral tubulin polymerizing base buffer for solution made from 80 mM K-PIPES, pH 6.8, 1 mM MgSO4, 1 mM EGTA, stored at 4°C for upto one year.
Permanent double-sided tape	3M - Scotch	34-8724-5691-7	For experimental chambers
Petri dish	Fisher	FB0875713	For humid chamber
PIPES	Sigma	P7643-100G	Tubulin buffer base ingredient
Pluronic-F127	Sigma	P2443-250G	A block-copolymer with two hydrophilic polyethylene oxide (PEO) blocks on the ends and a hydrophobic center block of polyphenylene oxide (PPO) hydrophobic surface coating we use to prevent protein binding to the surface. Pluronic-F127 is purchased as a powder (Sigma) and dissolved to a 5% (w/v) solution in ddH2O overnight. Once dissolved,

			the solution can be stored at room temperature for upto one year.
Polyethylene Glycol	Affymetrix Inc	19966 500 GM	A crowding agent used to create depletion forces to bring tactoids to the surface and help to organize microtubules. We create a 5% (w/v) solution of 100 kDa PEG in PEM80. The solution is stored in 4°C for upto one year and placed on the rocker before use. It is viscous, so we recommend using a positive displacement pipette to work with it.
Positive displacement pipette	Eppendorf		For pipetting viscous liquids
Racks for coverslips	Electron Microscopy Sciences	72240	For preparing experimental chambers - treating cover glasses
Refrigerator 4°C	Fisherbrand		For storing reagents
Tubulin Labeled	Cytoskeleton	TL590M	lyophilized rhodamine-labeled tubulin from pig brain is purchased (Cytoskeleton) and stored in -80°C until hydrated in PEM80 and used for upto one year.
Tubulin protein	Cytoskeleton	T240	lyophilized tubulin 99% pure unlabeled from pig brain is purchased (Cytoskeleton) and stored in -80°C until hydrated in PEM80 and used for upto one year.
UV-Ozone	Jelight	Model 342	For preparing experimental chambers - treating cover glasses
Stackreg	Biomedical Imaging Group	http://bigwww.epfl.ch/thevenaz/stackreg/	plugin for registering time series data to remove drift
Turboreg	Biomedical Imaging Group	http://bigwww.epfl.ch/thevenaz/turboreg/	plugin for registering time series data to remove drift