

Reconstitution of Actin-Based Motility with Commercially Available Proteins

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Materials

Name	Company	Catalog Number	Comments
Actin, rabbit muscle, Alexa Fluor 488 conjugate	Invitrogen (ThermoFisher Scientific)	A12373 (recently discontinued)	This product can be replaced with ATTO-488 actin from Hypermol.
Actin, rabbit muscle, ATTO-488	Hypermol	8153	
Actin, rabbit skeletal muscle	Cytoskeleton	AKL99	
Arp2/3 complex	Cytoskeleton	RP01P	
ATP	Sigma	A7699	
BioSpectrometer, basic	Eppendorf	035739	
Bradford Reagent	Bio-Rad	500-0006	
BSA, high quality	Sigma	A3059	
BSA standard 2 mg/mL (Pierce)	Thermo Scientific	23209	
Capping protein (a1b2, mouse recombinant)	Home-purified (Reference 13)		This product will soon be commercially available from Cytoskeleton.
Capping protein (a1b2, human recombinant)	Hypermol	8322	
Cube, GFP: U-MNIBA3 or U-MWB2	Olympus	discontinued	Any GFP cube, adapted to the microscope being used, can be used.
Dry block, agitating: ThermoMixer C (refrigerated)	Eppendorf	035963	
** with SmartBlock, 24 microtubes 2 mL	Eppendorf	035969	
Gelsolin (human recombinant, His-tagged)	Cytoskeleton	HPG6	
Lanolin	Sigma	49909	
Microcentrifuge 5427R + rotor	Eppendorf	934126	
Microscope, upright: BX51	Olympus	discontinued	Any epifluorescence upright microscope equipped with phase contrast optics can be used.
Microscope, inverted: IX70	Olympus	discontinued	Any epifluorescence inverted microscope equipped with phase contrast optics can be used.
Paraffin	Sigma	76244	

Petroleum jelly: Vaseline	Sigma	16415	
Pipettes Research Plus	Eppendorf		Gilson pipettes don't work as well for delivery of very small volumes (0.5 μ L for example).
**10 μ L		933954	
**2.5 μ L		933953	These two sizes are essential, but the use of high-quality pipettes (a full Research Plus set for example) is recommended.
Polystyrene carboxylate beads	Polysciences		
**approx. 1 μ m diameter		08226	
**approx. 4.5 μ m diameter		17140-5	
Profilin 1 (human recombinant, untagged)	Cytoskeleton	PR02	
SpVCA (human WASP pVCA domain, N-ter His-tag, C-ter Streptavidin tag)	Home-purified (Reference 14)		This product will soon be commercially available from Cytoskeleton.
VCA (human WASP VCA domain, GST-tagged)	Cytoskeleton	VCG03	