

Materials List for:

Assessment of *Dictyostelium discoideum* Response to Acute Mechanical Stimulation

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Materials

Name	Company	Catalog Number	Comments
Reagents			
Dextrose	Fisher	D16-1	Use to prepare HL-5 media and SM plates (steps 1.1, 1.2)
Proteose peptone	Fisher	DF0120-17-6	Use to prepare HL-5 media (step 1.1)
Yeast extract	Fisher	50-550-445	Use to prepare HL-5 media and SM plates (steps 1.1, 1.2)
Na ₂ HPO ₄ ·7H ₂ O	Fisher	S373-500	Use to prepare HL-5 media, and 10X Phosphate Buffer (steps 1.1, 1.3)
KH ₂ PO ₄	Fisher	P386-500	Use to prepare HL-5 media, SM plates, and 10X Phosphate Buffer (steps 1.1-1.3)
Streptomycin (dihydrostreptomycin sulfate)	Fisher	ICN10040525	Use to prepare HL-5 media (step 1.1)
Peptone (Bacto)	Fisher	DF0118-17-0	Use to prepare SM plates (step 1.2)
K ₂ HPO ₄	Fisher	P288-100	Use to prepare SM plates (step 1.2)
Agar	Fisher	BP1423-500	Use to prepare SM plates (step 1.2)
MgSO ₄	Fisher	M65-500	Use to prepare DB Buffer (step 1.4)
CaCl ₂	Fisher	C79-500	Use to prepare DB Buffer (step 1.4)
Caffeine	Fisher	O1728-500	Use to prepare caffeine (step 1.5)
cAMP (EMD Millipore Calbiochem Adenosine 3 ft.,5 ft.-cyclic Monophosphate, Sodium Salt)	Fisher	11-680-1100MG	Use to prepare cAMP (step 1.6)
Folic acid	Sigma	F7876	Use to prepare folic acid (step 1.7)
Tris base	Fisher	BP152-500	Use to prepare 3X sample buffer (step 1.8)
Sodium dodecyl sulfate (SDS)	Fisher	BP166-500	Use to prepare 3X sample buffer (step 1.8)
Glycerol	Fisher	G33-500	Use to prepare 3X sample buffer (step 1.8)
Dithiothreitol (DTT)	Bio-Rad	1610610	Use to prepare 3X sample buffer (step 1.8)
Bromophenol blue	Bio-Rad	1610404	Use to prepare 3X sample buffer (step 1.8)

NaF	Fisher	S299-100	Use to prepare sample buffer with protease and phosphatase inhibitors (step 1.8)
Na ₃ VO ₄	Fisher	50-994-911	Use to prepare sample buffer with protease and phosphatase inhibitors (step 1.8)
Sodium pyrophosphate decahydrate	Fisher	S390-500	Use to prepare sample buffer with protease and phosphatase inhibitors (step 1.8)
Complete EDTA-free protease inhibitor cocktail (Roche)	Sigma	11873580001	Use to prepare sample buffer with protease and phosphatase inhibitors (step 1.8)
Latrunculin A	Enzo Life Sciences	BML-T119-0100	Use to prepare Latrunculin A (step 1.9)
4-15% Tris-HCl polyacrylamide gel	Bio-Rad	3450029	Use for immunoblotting (step 3.3)
Polyvinylidene fluoride membrane	Bio-Rad	1620177	Use for immunoblotting (step 3.3)
Phospho-PKC (pan) (zeta Thr410) (190D10) Rabbit antibody	Cell Signaling	2060	Primary antibody for use in immunoblotting (step 3.3)
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP antibody	Cell Signaling	4370	Primary antibody for use in immunoblotting (step 3.3)
Rabbit IgG HRP Linked Whole Ab (from Donkey)	GE Healthcare	NA934-100UL	Secondary antibody for use in immunoblotting (step 3.3)
Enhanced chemiluminescence substrate (Clarity Western ECL Substrate)	Bio-Rad	1705060	Use for immunoblotting (step 3.3)
Stripping buffer (Restore Plus Western blot stripping buffer)	Pierce	PI46430	Use for immunoblotting (step 3.3)
Coomassie Brilliant Blue	Fisher	PI20278	Use for immunoblotting (step 3.3)
<i>K. aerogenes</i> strain (non-pathogenic)	Dicty Stock Center (dictybase.org)		
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Materials and Equipment			
Orbital shaker (model G-33, or a comparable alternative)	New Brunswick Scientific		Use to grow <i>K. aerogenes</i> (step 2.1.1), and to basalate and stimulate <i>D. discoideum</i> cells (step 3). The radius of gyration for this shaker is 9.5 mm.
10 cm Petri dish	Fisher	FB0875712	Use to prepare SM plates (step 1.2)
Hemocytometer	Fisher	02-671-51B	Use to count <i>D. discoideum</i> cells (step 2.1.2)
35 mm dish (Corning Falcon Easy-Grip Tissue Culture Dish)	Fisher	08-772A	Use to plate <i>D. discoideum</i> cells for mechanical stimulation followed by cell lysis (step 3.1.1)
Polystyrene cup (5 mL)	VWR	13915-985	Use to stimulate <i>D. discoideum</i> cell suspension with a chemoattractant (step 3.2.2)
Fluidic unit with pump (Ibidi Pump System)	Ibidi	10902	Use to deliver mechanical stimulation to <i>D. discoideum</i> cells in a channel (steps 4.1, 4.2, and 5)
Slide with a channel (μ -Slide III 3in1 ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized)	Ibidi	80316	Use to plate <i>D. discoideum</i> cells for mechanical stimulation delivered by a fluidic device (steps 4.1, 4.2, and 5)
50 mL reservoirs (Ibidi Reservoir Holder for Fluidic Unit)	Ibidi	10978	Use to setup the fluidic device for delivering mechanical stimulation

			to <i>D. discoideum</i> cells in a channel (steps 4.1, 4.2, and 5)
Lines for the fluidic unit (Perfusion Set YELLOW-and-GREEN)	Ibidi	10964	Use to setup the fluidic device for delivering mechanical stimulation to <i>D. discoideum</i> cells in a channel (steps 4.1, 4.2, and 5). Note: the two lines can also be cut from the 1.6 mm tubing (catalog number 10842).
Line for the drain (1.6 mm ID tubing)	Ibidi	10842	Use to setup the fluidic device for delivering mechanical stimulation to <i>D. discoideum</i> cells in a channel (steps 4.1, 4.2, and 5).
Zeiss Observer.Z1 inverted microscope equipped with a 40X/1.3 oil objective and a 20X/0.3 air objective (or a comparable alternative)	Zeiss		Used to image <i>D. discoideum</i> cells with phase-contrast or fluorescence illumination (steps 4 and 5)
UltraView spinning disk confocal microscope equipped with a 40x/1.25–0.75 oil objective (or a comparable alternative)	Perkin-Elmer	DM 16000	An alternative used to image <i>D. discoideum</i> cells (step 4.1.4)
FemtoJet Microinjector (Eppendorf; model 5247, or a comparable alternative) with Eppendorf InjectMan	Eppendorf	5252000021D and 5192000027	Use to deliver mechanical stimulation to <i>D. discoideum</i> cells by a micropipette (step 4.3). Note: catalog number are for the newest version of the equipment (4i).
Micropipette (Femtotip; 1 µm outside diameter, 0.5 µm inside diameter)	Eppendorf	930000035	Use to deliver mechanical stimulation to <i>D. discoideum</i> cells by a micropipette (step 4.3)
Microloader tips (Eppendorf Femtotips Microloader Tips for Femtojet Microinjector)	Eppendorf	5242956.003	Use to fill the micropipette (femtotip) with buffer (step 4.3.1)
1-well chamber (Thermo Scientific Nunc Lab-Tek Chambered Coverglass)	Fisher	12-565-472	Use to plate <i>D. discoideum</i> cells for mechanical stimulation delivered by a micropipette (step 4.3.2)
8-well chamber (Thermo Scientific Nunc Lab-Tek II Chambered Coverglass)	Fisher	12-565-338	Use to plate <i>D. discoideum</i> cells for mechanical stimulation delivered by bulk buffer addition (step 4.4.1)
Name	Company	Catalog Number	Comments
Software			
Image Analysis Software (Fiji)	NIH	https://fiji.sc/	Use to quantify response to mechanical stimulation (step 4.5)
Migration analysis software (Tracking Tool PRO)	Gradientech	http://gradientech.se/tracking-tool-pro/	Use to quantify cell speed and persistence (step 5.4)