

Materials List for:

Tissue Triage and Freezing for Models of Skeletal Muscle Disease

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URL: <https://www.jove.com/video/51586>

DOI: [doi:10.3791/51586](https://doi.org/10.3791/51586)

Materials

Name	Company	Catalog Number	Comments
For tissue freezing			
Liquid nitrogen dewar with a liquid withdrawal device	Custom Biogenic Systems	Lab-5 Series	Any other liquid nitrogen dewar could substitute.
Cold-conductive container	Fisher	02-583A	
Wide insulated container capable of holding liquid nitrogen	Nalgene	4150-2000	
Oakton Temp 10 Thermocouple Thermometer	Thomas Scientific	1228Y01	Optional, but can be very useful in identifying the point at which isopentane is freezing.
For single fiber functional testing			
Petri dish	Fisher Scientific	08-757-11	
Sylgard coating	Ellsworth Adhesives	3-6636	
Dissection stereomicroscope	Harvard Apparatus	693101	Any other dissection microscope could substitute.
For cell culture			
Sterile laminar flow hood	Thermo Scientific	51022485	Any other cell culture hood could substitute.
For tissue freezing			
Plastic bags/containers	Nasco	B01009WA	
Thermal safety gloves	Denville	G4162	
Face shield	Fisher Scientific	S47640	
Long forceps	Fisher Scientific	12-460-807	
Marker	Staples	125328	
For cell culture			
Sterile 50 ml conical tubes	Denville	C1060-P	
PES filter unit, 500 ml, 0.22 µm	Denville	F5227	
Sterile forceps	Fisher Scientific	644320	
Ice packs	Fisher Scientific	NC9909223	
Insulated styrofoam box	Polar Tech	207F	
Packing tape	Staples	795570	

Tissue freezing			
Liquid nitrogen	Airgas	NI NF160LT350	Store liquid nitrogen in containers designed for low-temperature liquids
Isopentane (2-methylbutane)	Sigma	M32631-4L	Store Isopentane in a flammable materials cabinet.
EM fixative (choose one)			The buffers without fixative should also be available, as EM-fixed tissues should be transferred from fixative to buffer after several hours or days if they are not immediately processed for EM.
2.5% glutaraldehyde in 0.1M cacodylate buffer	Electron Microscopy Sciences	16537-15	
Karnovsky's fixative (3% glutaraldehyde + 2% paraformaldehyde in 0.1M phosphate buffer)	Electron Microscopy Sciences	15732-10	
For functional studies (if desired)			
Relaxing solution, containing (in mmol/L)			
40 BES	Sigma	B9879	
10 EGTA	Sigma	E4378	
6.56 MgCl ₂	Sigma	M8266	
5.88 Na-ATP	Sigma	A3377	
1.0 DTT	RPI	D11000	
46.35 K-propionate			Make a solution by mixing propionic acid (Sigma P1386) and KOH (Fisher P250) 1:1
15 creatine phosphate, pH 7.0	Sigma	P7936	
0.4 leupeptin	Calbiochem	10895	
0.1 PMSF	Sigma	P7676	
Skinning solution, containing			
Relaxing solution (See Above)			
0.5% Triton X-100	Sigma	T8787	
Indicating silica granules			
Flat pieces of cork (1 x 1 inch)	Electron Microscopy Sciences	63305	
Glycerol	Sigma	G2025	
For cell culture (if desired)			
Complete Growth Medium, containing (for 500 ml)			Sterilize by filtering the solution through a 500 ml 0.22 µm PES filter unit. Store at 4°C and use within 1 month.
395 ml of high glucose Dulbecco's Modified Eagle's Medium (DMEM)	Sigma	D5671	
100 ml of fetal bovine serum (FBS)	Sigma	F6178	
5 ml of 100X Penicillin-Streptomycin-Glutamine (PSG)	Sigma	G1146	
See the materials safety data sheet (MSDS) for all other reagents for storage specifications			