

Materials List for

Fiber Type and Subcellular-Specific Analysis of Lipid Droplet Content in Skeletal Muscle

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jove.com/video/63718

Materials

Name	Company	Catalog Number	Comments
Equipment			
AxioCam 506 mono 6 Mpix camera	Zeiss		
AxioCam MRm 1.4MPix CCD camera	Zeiss		
Chemical hood	Potteau Labo	EN-14175	
Confocal microscope	Zeiss	LSM800	
Cork discs (ø 20 mm, 3 mm thick)	Electron Microscopy Sciences	63305	
Cryo-Gloves	Tempshield	16072252	
Cryostat	Thermo Scientific	Microm Cryo Star HM 560	
Dissecting Stereo Microscope SMZ745	Nikon		
Dry Ice			
Dumont Forceps	F.S.T	11295-10	
Epifluorescence microscope	Zeiss	Axiolmage-Apotome Z1	
Extra Fine Bonn Scissors	F.S.T	14084-08	
FisherBrand Disposable Base Molds (0.7 x 0.7 cm)	ThermoFisher	22-363-552	Used to cut a piece to hold the muscle on the cork for freezing
Glass petri dish (H 25 mm, ø 150 mm)	BRAND Petri dish, MERK	BR455751	Used to place the muscles on ice during dissection
ImmEdge Hydrophobic barrier PAP Pen	Vector Labs	H-4000	Used to create an hidrophobic barrier around the muscle sections
Incubator	MMM Medcenter	Incucell 707	
Microscope Cover Glasses (24x50 mm)	Assistent	40990151	
Microscope Slide Boxes	Kartell	278	Used as humid chambers for immunohistochemistry
Neck holder	Linie zwo	SB-035X-02	Used as strap to hold the stainless steel tumbler
No 15 Sterile Carbon Steel Scalpel Blade	Swann-Morton	0205	

Paint brushes	Van Bleiswijk	Amazon B07W7KJQ2X	Used to handle cryosections
Permanent Marker Pen Black	Klinipath/VWR	98307-R	Used to label slides
Pierce Fixation Forceps	F.S.T	18155-13	
Polystyrene Box			H 12 cm x L 25 cm x W 18 cm, used as a liquid nitrogen container and to transport the samples to the cryostat
Scalpel Handle, 125 mm (5"), No. 3	Aesculap	BB073R	
Stainless Steel Cup 10oz	Eboxer	B07GFCBPFH	Tumbler to fill with isopentene for muscle freezing
Superfrost Ultra Plus slides	ThermoFisher	J1800AMNZ	
Surgical tweezers 1/2 teeth	Medische Vakhandel	1303152	Also called "Rat teeth tweezers"
Vannas Spring Scissors - 3 mm Cutting Edge	F.S.T	15000-00	
Weighing boats	VWR international	611-2249	
Whole-Slide Scanner for Fluorescence	Zeiss	Axio Scan.Z1	
Reagents			
Alexa Fluor 405 Goat Anti-Mouse IgG2b	Sigma-Aldrich	SAB4600477	Used at a final concentration of 1:500
Alexa Fluor 488 Goat Anti-Mouse IgG1	ThermoFisher	A-21121	Used at a final concentration of 1:500
Alexa Fluor 568 Goat Anti-Mouse IgM	Abcam	ab175702	Used at a final concentration of 1:1,000
Alexa Fluor 647 goat anti rat-IgG (H +L) secondary antibody	ThermoFisher	A-21247	Used at a final concentration of 1:500
BODIPY-493/503 (4,4-difluoro-1,3,5,7,8-pentametil-4-bora-3a,4a-diaza-s-indaceno)	ThermoFisher	D3922	Used at a final concentration of 1 µg/mL
BODIPY-558/568 C12 (4,4-Difluoro-5-(2-Thienyl)-4-Bora-3a,4a-Diaza-s-Indacene-3-Dodecanoic Acid)	ThermoFisher	D3835	Used at a final concentration of 1 µg/mL
DAPI (4',6-diamidino-2-phenylindole)	ThermoFisher	D1306	Used at a final concentration of 0.5 µg/mL
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich	D-8418	Used to solve Bodipy for the 1 mg/mL stock solution. CAUTION: Toxic and flammable. Vapors may cause irritation. Manipulate in a fume hood. Avoid direct contact with skin. Wear rubber gloves, protective eye goggles.
Formaldehyde solution 4%, buffered, pH 6.9	Sigma-Aldrich	1004969011	CAUTION: May cause an allergic skin reaction. Suspected of causing genetic defects. May cause cancer. Manipulate in a fume hood. Avoid direct contact with skin. Wear rubber gloves, protective eye goggles.
Isopentane GPR RectaPur	VWR international	24872.298	CAUTION: Extremely flammable liquid and vapor. May be fatal if swallowed and enters airways. May cause drowsiness or dizziness. Repeated exposure may cause skin dryness or cracking. Wear protective gloves/protective clothing/eye protection/face protection.
Liquid Nitrogen			CAUTION: Extremely cold. Wear gloves. Handle slowly to minimize boiling and splashing and in well ventilated areas. Use containers designed for low-temperature liquids.

Mouse on mouse Blocking Reagent	Vector Labs	MKB-2213-1	Used at concentration of 1:30
Myosin heavy chain Type I (BA-D5-s Primary Antibody) Gene: MYH7, monoclonal bovine anti mouse IgG2b	DSHB University of Iowa	BA-D5-supernatant	Used at a final concentration of 1:10
Myosin heavy chain Type IIA (SC-71-s Primary Antibody) Gene: MYH2, Monoclonal bovine anti mouse IgG1	DSHB University of Iowa	SC-71-supernatant	Used at a final concentration of 1:10
Myosin heavy chain Type IIX (6H1-s Primary Antibody), Gene: MYH1, Monoclonal rabbit anti mouse IgM	Developmental Studies Hybridoma Bank, University of Iowa	6H1-supernatant	Used at a final concentration of 1:5
Normal Goat Serum (NGS)	Vector Labs	S-1000	
PBS 0.1 M			Commonly used on histology laboratories
ProLong Gold Antifade Mountant	Invitrogen	P36930	
Rat anti-Laminin-2 (α -2 Chain) primary antibody (monoclonal)	Sigma-Aldrich	L0663	Used at a final concentration of 1:1,000
Tissue-Tek O.C.T compound	Sakura	4583	
Software			
Adobe Illustrator CC	Adobe Inc.		Used to design the figures
Adobe Photoshop	Adobe Inc.		Confocal software
BioRender	https://biorender.com/		Used to design the figures
Fiji/ImageJ	https://imagej.net/software/fiji/		Used to analyse the acquired images
Microsoft PowerPoint	Microsoft		Used to reconstruct the histology of the whole muscle after scanning the fiber types
Zen Blue 2.6	Zeiss		Used to reconstruct the histology of the whole muscle after scanning the fiber types