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Collection and shipment of live skeletal muscle for RNA and cell isolation

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We are still developing and optimizing this protocol

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Abstract

Collecting and transporting fresh muscle tissue from a clinical site for experimentation at a remote location can be a logistical challenge. This protocol provides the procedures for collecting, preserving and shipping fresh skeletal muscle autopsy samples from the morgue to a remote laboratory site for downstream experiments requiring viable cells and
high-quality RNA such as single-cell RNA-seq.

Guidelines

Tissue collection for this protocol needs prior approval by the users' Institutional Ethics Board or equivalent ethics committee.

Materials

	Reagent Name	Description	Vendor	Manufacturer	Notes
	Organ Transplant Solution	Beltzer UW cold storage solution	Fisher Scientific, cat# NC2122383	Bridge to life	https://bridgetolife.com/belzer-uw-cold-storage-solution/
	RNAse Inhibitor	Protector RNase inhibitor	Millipore Sigma # RNAINH-RO	Roche # 3335402001	https://www.sigmaaldrich.com/US/en/product/roche/rnainhro?gclid=EAIaIQobChMI9e2O0ZeDgQMVcc7ICh3dKAagEAAYAiAAEgIPpPD_BwE
	Cryotubes	Corning™ Internally Threaded Cryogenic Vials	Fisher Scientific, # 03-374-21	Corning # 430488	https://www.fishersci.com/shop/products/corning-internally-threaded-cryogenic-vials-15/0337421?crossRef=337421#?keyword=337421
	Scale / balance (milligram units)	Laboratory grade balance; OHAUS Pioneer PX Precision Balances	Fisher Scientific, # 01-922-178	OHAUS # 30429848	https://www.fishersci.com/shop/products/pioneer-px-balance-26/01922178?keyword=true
	Small Foreceps	Small forceps for handling tissues	Fisher Scientific, # 13-812-211		
	Kimwipes		Fisher Scientific, Catalog # 06-666		
	Freezer packs	Sonoco ThermoSafe PolarPack Standard	Fisher Scientific, # 03-530-110		
	Insulated foam shipping kit	Insulated foam box fitted inside a cardboard shipping box, at least 12 × 12 x 11.5 inches	Uline, # S-13392		
	Ice Bucket or insulated foam box	Fisherbrand™ Polyurethane Ice Buckets	Fisher Scientfic, #02-591-45		
	Weigh boats	Small (1.75″ x 1.75″ x 0.24″) and medium			

	Reagent Name	Description	Vendor	Manufacturer	Notes
		weigh boats (3.25″ x 3.25″x 1″)			
	Razorblade or surgical knife				
	Wet Ice				
	Packaging Tape				
	Shipping label				

Troubleshooting

Sample collection & preparations

1 At sample collection site, communicate and schedule with mortician to come pick up the muscle autopsy sample, ensuring samples be collected 48 hours or less postmortem.

Note

The smaller the postmortem window prior to sample collection the better. We have tested up to 72 hours postmortem, obtaining usable RNA quality if isolated within 48 hours of being stored in chilled preservation mix.

Note

Unless restricted, request to collect quadricep muscle as it is the most commonly biopsied tissue in muscular dystrophy patients and may allow for direct comparisons.

Note

Part of the autopsy sample may need to be snap frozen and sectioned for histological examination (see sample freezing protocol).

1.1 Record relevant details about the sample such as: the site name, subject ID, age of donor when sample collected, sex assigned at birth, gender, ethnicity, cause of death, medications and medical history, BMI, postmortem time, muscle group, and collection date. These details may be relevant to downstream experiment.

2 Collect one gram (1000 mg) or more of quadricep muscle sample such that it does not dry during the procedure*.

3 Place the harvested muscle into a 50 mL conical tube half filled with chilled transplant solution On ice . Place the tube in a 1.2-gallon (or larger) polyurethane or insulated foam bucket full of wet ice. Close the lid and quickly transport to the lab.

Note

The muscle can also be transported to the lab in a tube of chilled saline OR a saline soaked piece of gauze placed on ice, but using transplant solution is recommended.

Note

If the muscle gets dried at any point during processing, it will lead to histological artifacts, cell death and poor-quality RNA (RIN <7).

4 In the laboratory and prepare to weigh the muscle sample by placing a small or medium empty weigh boat on a laboratory scale, and tare.

5 Working quickly, remove the wet muscle using a pair of forceps from the tube and absorb excess transplant solution from the muscle tissue by wicking on clean Kim wipes. Further, remove the excess liquid by thoroughly dabbing the tissue on a fresh Kim Wipe.

6 Immediately transfer the muscle onto the weigh boat, record the weight and moisten the muscle by placing it into the original tube of chilled transplant solution.

7 Take the wet muscle piece back out of the transplant solution and place on a medium weigh boat.

8 Using a clean razor blade, roughly divide the muscle into pieces smaller than 250 mg and place each piece into an individual cryotube.

Note

e.g., 1,000 mg autopsy sample will be divided into 4 pieces; larger pieces prevent the transplant solution from diffusing and preserving the entire muscle piece.

Note

Note: At this stage, a few of the divided muscle pieces can be snap frozen as needed [link to frozen muscle transport protocol] for histological analysis, spatial RNA-seq or single nuclear RNA-seq [link to Karim's protocol]

Preserving and shipping samples

9 Prepare 500 µL of preservation buffer by mixing 497.5 µL of chilled transplant solution and 2.5 µL of RNAse inhibitor ([M] 40 U/mL stock) for a final concentration of [M] 0.2 U/mL per piece of muscle into individual cryotube.

10 Submerge each of the divided muscle pieces into individual cryotube with preservation buffer. Close the screw caps, label with sample ID and date, and place on wet ice.

11 Prepare for shipping by placing 1-2 freezer packs into an insulated foam box fitted inside a cardboard shipping box and add wet ice on top. Place the sample tubes into the wet ice.

12 Close the foam and cardboard boxes and tape the seams shut using packaging tape. Place label and send the package for overnight shipping.

Expected result

RNA quality testing

We have tested the RNA quality of human autopsy samples collected at various postmortem intervals (6-72 hours) and kept in preservation mix for 24hrs (after overnight shipping) and 48 hours on ice (24 hours after receiving shipment at remote site). The samples were snap frozen in liquid nitrogen cooled isopentane at the sample collection site (0hr, +control), and at the remote site after 24 hours and 48 hours in saline + RNA inhibitor or preservation mix. The frozen muscles were sectioned on a cryostat and RNA extracted using a Zymo RNA isolation kit. The whole muscle RNA quality was assessed on an Agilent TapeStation.

Human samples in the preservation mix had RIN numbers comparable or one lower than samples immediately snap frozen prior to shipping (RIN ~8). The samples in saline + RNAse inhibitor had similar quality RNA but trended a bit lower. E.g., Samples immediately frozen have RIN # ~8, samples in preservation mix after 24-48 hours RIN > 7. This preserved fresh tissue are suitable for live cell isolations for downstream experiments.

Our lab has used mouse limb muscle samples preserved in this mix for 24 hours and continued to digest, and FACS isolate live muscle cells. We were able to obtain 150-300K live cells from a gram of skeletal muscle tissue. We were able to isolate high quality RNA was isolated from these cells (RIN > 7).