



Pathology Core Protocol

Protocol Section:	General Procedures	Policy No:	P-CMHD1-MUSC-00
Protocol Subject:	Muscle Sample Collection	Effective Date:	
		Date Reviewed:	6 January 2003
		Date Revised:	May 18, 2004

Muscle Sample Collection and Processing for EM

Universal EM Fixative:

Sodium phosphate monobasic	11.6g
Sodium hydroxide pellets	2.7g
50% glutamate	20%
37% formaldehyde	100ml
Distilled water	1000ml

1. Immediately after euthanasia, recover the diaphragm, intracostal, limb muscle, etc. from the mouse and using iris scissors or a scalpel blade, cut into several pieces (approximately 1cm x 1cm)
2. Place one piece in 10% neutral buffered formalin if paraffin embedded sections are required
3. Using an eye dropper, place several drops of Universal Fixative (UF) on a red wax cutting board to create a puddle
4. Place one piece of muscle in the puddle of UF and using a new scalpel blade or razor blade, mince into 1mm x 1mm cubes
5. Using fine needle nose forceps transfer the cubes of diaphragm to the vial of UF. Incubate the fixative for at least one hour at 4⁰C and submit for post-fixing and processing

Muscle Sample Collection for Frozen Sections

If the cross-sectional diameter of the muscle sample is >2 mm:

1. Immediately after euthanasia, recover the diaphragm, intracostal, limb muscle, etc. from the mouse and place the strip of muscle longitudinally on an applicator stick
2. Immerse the applicator stick with the sample in isopentane, cooled according to the following:
 - Add isopentane to a beaker and lower the beaker into liquid nitrogen until the isopentane just starts to solidify around the edges
 - As soon as the isopentane starts to solidify around the edges, pull the beaker out of the liquid nitrogen and place the muscle into the isopentane immediately. Leave the muscle sample in the isopentane for one minute (until the muscle is frozen) and then leave the muscle on the stick for storage at -80⁰C
 - To package for storage, break off the stick and place it in an eppendorf or cryovial
3. Store the sample at -80⁰C until cryosectioning

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If the cross-sectional diameter of the muscle sample is <2 mm:

1. Immediately after euthanasia, recover the diaphragm, intracostal, limb muscle, etc. from the mouse and lay the sample in longitudinal section in a cryomold filled with OCT
2. Immerse the mold in liquid nitrogen
3. Store the sample at -80°C until cryosectioning

Ensure that the person doing the sectioning is aware of the longitudinal section in the mold so that the sample can be placed on the chuck appropriately for cross sections

Reference: The Hospital for Sick Children, the Department of Pathology, Division of Neuropathology

Issued by Lab Manager: _____ **Date:** _____

Approved by Facility Management: _____ **Date:** _____

4.