MAYO
Mayo Medical Laboratories

Muscle Specimen Preparation Instruction Sheet

Directions for Preparing Muscle Specimens for Histochemical Study at Mayo

The muscle biopsy should be obtained from a muscle that is definitely affected, but not so severely affected that much of it is replaced by fatty or fibrous connective tissue. This usually means a -1 to -2 rating on the Mayo Clinic manual muscle testing scale, or a +4 rating on the MRC scale. Also, the involved muscle should not have been previously traumatized by injections or by EMG studies. Typically, the triceps, biceps or vastus lateralis is chosen. An approximately **1 cm X 0.5 cm specimen should be obtained,** and dissected with minimum trauma along the long axis of the muscle fibers. **Due to the nature of the testing to be done, do not use electrocautery or a muscle clamp in removing the specimen. Also, do not use a mounting medium such as OCT when freezing the specimen.**

If you should receive a muscle biopsy in a clamp or another apparatus, remove the specimen from it immediately. If the specimen is larger than 1 x 1.5 cm in size, subdivide it into smaller pieces and freeze the pieces individually. The specimen should be blotted with an absorbent towel to extract excess moisture before flash-freezing.

In order to optimize the quality of the frozen specimen, **place the specimen container on dry ice or in a -70° freezer** prior to flash-freezing.

Two methods are recommended for flash-freezing:

METHOD 1: SLURRY METHOD

Wrap solid dry ice in a towel, pulverize it with a hammer, and then pour the powder to fill a 200 mL beaker. Slowly add 100% alcohol or acetone and stir the mixture. At least 80% of the total volume of the slurry should consist of dry ice and only 20% of alcohol or acetone. When the slurry nearly stops bubbling, its temperature has fallen to about -70° C, and it is suitable for flash-freezing. Keep adding dry ice as needed to reach this point. The final product should resemble a "snowcone." Hold the biopsy specimen with a forceps, and plunge it into the slurry quickly. Swirl the specimen in the slurry for 10–15 seconds, remove it, and quickly blot dry with absorbent towel to remove excess alcohol/acetone. Immediately place tissue in specimen container that has been pre-chilled on dry ice. Place cover on container. Puncture lid to allow excess alcohol/acetone to evaporate. Keep specimen frozen from this point on. **Note:** a well-frozen specimen should have a white chalky color. *Avoid prolonged immersion in the quenching mixture, as the specimen becomes permeated with alcohol or acetone which inhibits enzyme studies by histochemical methods.* The specimen can be temporarily stored at -70° C, but must not thaw between the time of initial freezing and shipment. Never place the frozen specimen in an unchilled container as the specimen will thaw before it freezes again.

METHOD 2: ISOPENTANE – LIQUID NITROGEN FREEZING

Take a Nalgene® or metal beaker, add 50–100 mL of isopentane (2-methylbutane). Suspend the beaker in a bath of liquid nitrogen and wait until the isopentane freezes to a white, chalky substance. Remove the beaker from the nitrogen bath. Hold the muscle specimen with a forceps, and press it against the solid isopentane. The isopentane will start to thaw as soon as it contacts the warmer muscle specimen. At this point, it is important to swirl the specimen around so that it will continue to equilibrate with the colder, frozen isopentane. The total freezing period should take approximately 10–15 seconds. After removing the specimen from the isopentane, quickly blot it dry with an absorbent towel to remove excess isopentane. Immediately place tissue in specimen container that has been pre-chilled on dry ice. Place cover on container. Puncture lid to allow exess isopentane to evaporate. Keep specimen frozen from this point on. **Note:** a well-frozen specimen should have a white chalky color. It also can be temporarily stored at -70° C but must not thaw between the time of initial freezing and shipment. Never place the frozen specimen in an unchilled container as the specimen will thaw before it freezes again.

PATIENT INFORMATION

To assist in interpretation of the specimen and for our permanent records, fully complete the "Muscle Histochemistry Patient Information Sheet." Attach any pertinent studies, such as the serum creatine kinase level, electromyographic studies, and suspected clinical diagnosis. Be sure to indicate the address or addresses where results should be sent. Place this information in the shipping box with the specimen. **Biopsies received without this information will be held until the appropriate information is obtained.**

SHIPPING

- **Use Muscle Biopsy Shipping Kit (Supply T541)**
- **Shipment via courier service is recommended.**
- **Contact courier 1 day before shipping to alert them to bring extra dry ice.** If not shipping by courier, specimen must be packed in 10-20 lbs of dry ice to last for a minimum of three days. Ship via an express overnight delivery service.
- **Send Monday, Tuesday, Wednesday only.** If biopsy is taken on Thursday or Friday, flash freeze using one of the above methods and store at -70°C until it can be sent the following Monday. The container should prominently indicate that it contains frozen biological material.

Shipping address:
Attn: Muscle Sample Enclosed
Mayo Medical Laboratories
3050 Superior Drive NW
Rochester, MN 55901

QUESTIONS

If there are questions related to specimen preparation,contact:
Mayo Clinic Muscle Laboratory
Phone: 507-284-5100
Fax: 507-284-5831

To review clinical problems with the clinician before the biopsy is preformed, contact:
Andrew G. Engel, M.D.
Mayo Clinic
Phone: 507-284-5100

Phone 1-800-533-1710 to order Muscle Biopsy Shipping Kits (Supply T541)