MAYO CLINIC LABORATORIES

Muscle Biopsy Specimen Preparation Instructions

Patient Information

To prevent delays and enhance accuracy of the interpretation, complete the Muscle Histochemistry Patient Information (T361 or included in the kit). Include any pertinent information, such as the suspected clinical diagnosis, serum creatine kinase level, results for collagen vascular disease enzymes, electromyographic (EMG) studies, and specific neurologic findings.

Instructions for Collecting and Sending Muscle Biopsy Specimens

Muscle Biopsy Collection Requirements and Precautions

1. Obtain biopsy 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 3 to 4 rating on the MRC scale.
   - The involved muscle **should not** have been previously traumatized by injections or by EMG studies.
   - Typically, the triceps, biceps or vastus lateralis is chosen.
2. Biopsy should be approximately 1.5 cm × 0.5 cm, and dissected with minimum trauma along the long axis of the muscle fibers. If extra studies are needed, then another piece of similar size should be obtained.
   - **Do not** use electrocautery or a muscle clamp in removing the specimen.
   - If a muscle biopsy is received in a clamp or another apparatus, remove the specimen from it immediately.
3. Freeze biopsy as soon as possible to limit the degradation of tissue.
4. Transport the specimen to the lab for freezing. Place specimen in wet gauze moistened by saline, in a bag, and place on wet ice.

Freezing Method – Utilize 1 of the 2 approved methods of freezing below.

Note: Freeze the muscle specimen so all fibers are oriented in the same direction.

- The specimen should be blotted with an absorbent towel to extract excess moisture before flash-freezing.
- **Do not** use a mounting medium such as OCT when freezing the specimen.
- **Do not** leave forceps impressions in the muscle.
- **Do not** use pin or needles, tongue depressors, or a muscle clamp.
- **Do not** wrap muscle in tin foil.
- A well-frozen specimen should have a white, chalky color.
- Never place the frozen specimen in an unchilled container as the specimen will thaw before it freezes again.
- The specimen can be stored at -70 °C, but **must not** thaw between the time of initial freezing and shipment.
- **Do not** freeze then thaw then re-freeze again.

Method 1: Isopentane – Liquid Nitrogen Freezing (preferred)

1. Puncture lid of a screw cap specimen container to allow excess isopentane to evaporate.
2. **Place plastic screw-cap specimen container in -70 °C freezer or on dry ice to prechill.**
3. Add 50–100 mL of isopentane (2-methylbutane) to a Nalgene or metal beaker. 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. Carefully drop or set the muscle on the frozen isopentane while keeping the muscle fibers straight and running in the same direction.
4. The isopentane will start to thaw as soon as it contacts the warmer muscle specimen. At this point, it is important to swirl the beaker around so that the specimen will continue to contact the colder, frozen isopentane.
5. The total freezing period should take approximately 10–15 seconds.
6. After removing the specimen from the isopentane, immediately place the tissue in the container that has been prechilled on dry ice. **Do not** wrap in foil.
7. Place cover on container.
8. **Keep specimen frozen at -70 °C or on dry ice from this point on.**

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Muscle Biopsy Specimen Preparation Instructions (continued)

Method 2: Slurry Method

1. Puncture lid of a screw cap specimen container to allow excess alcohol/acetone to evaporate.
2. **Place plastic screw-cap specimen container in -70 °C freezer or on dry ice to prechill.**
3. Wrap solid dry ice in a towel, pulverize it with a hammer, and then pour the powder to fill a 200-mL beaker.
4. 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.
5. 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 "snow cone."
6. 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.

Note: Avoid prolonged immersion in the quenching mixture, as the specimen becomes permeated with alcohol or acetone, which inhibits enzyme studies by histochemical methods.

7. Immediately place tissue in specimen container that has been prechilled on dry ice. **Do not** wrap in foil.
8. Place cover on container.
9. **Keep specimen frozen at -70 °C or on dry ice from this point on.**

Packaging

1. Use Muscle Biopsy Kit (T541). Call 800-533-1710 or visit mayocliniclabs.com to order.
2. Place the transport vial with the flash-frozen specimen in the yellow frozen tissue bag with absorbent cloth and seal the bag.
3. Place the completed Muscle Histochemistry Patient Information form and any appropriate clinical history in the pocket of the bag.
4. Store the specimen in -70 °C freezer, dry ice, or liquid nitrogen until retrieved by the courier or ready to prepare for shipping to Mayo Clinic Laboratories.

Shipping

If using a courier service

- One day before shipping, contact Client Services at 800-533-1710 and ask for Global Logistics to alert them that a muscle biopsy will be shipped from your institution. The courier will bring extra dry ice for packaging.

If shipping via FedEx

- Send using **Priority** overnight delivery.
- Pack specimen in 8–10 lbs of pellet or small particle dry ice so the specimen is surrounded by the dry ice.
- Tape the box lid securely with packing tape.
- Shipping address:

  Mayo Clinic Laboratories
  Attn: Muscle Sample Enclosed
  3050 Superior Drive NW
  Rochester, MN 55905

Questions

For all questions, contact:

Mayo Clinic Laboratories
Phone 800-533-1710
Fax 507-284-5831

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