MAYO
Mayo Medical Laboratories

Muscle Biopsy Specimen Preparation
Instruction Sheet

Instructions for Muscle Biopsy Specimens for Histochemical Study at Mayo:

Muscle biopsy requirements and precautions

- 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 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.

Biopsy size and precautions

- An approximately 2 cm X 0.5 cm specimen should be obtained, and dissected with minimum trauma along the long axis of the muscle fibers. If extra studies are needed, then another piece or two of similar size should be obtained.
- 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.

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. This information must accompany the specimen. Completion of the study may be delayed until the appropriate information is obtained.
NOTE: If biopsy is taken on Thursday or Friday, flash freeze using one of the approved methods and store at -70°C until specimen can be sent the following Monday.

Freezing Method

- The specimen should be blotted with an absorbent towel to extract excess moisture before flash-freezing.
- Please utilize one of the two approved methods of freezing and store the specimen
  METHOD 1: SLURRY METHOD (See details on next page)
  METHOD 2: ISOPENTANE – LIQUID NITROGEN FREEZING (See details on next page)

Packaging

- Use Muscle Biopsy Shipping Kit (Supply T541) Call 1-800-533-1710 to order
- Place the transport vial with the specimen in the yellow frozen tissue bag with absorbent cloth and seal the bag
- Place the completed Muscle Histochemistry Patient Information Sheet and any appropriate clinical history in the outer pouch of the bag
- Store the specimen in -70ºC freezer until retrieved by courier or ready to prepare for shipping to Mayo Medical Laboratories

Shipping — SEND MONDAY, TUESDAY, WEDNESDAY ONLY

If using a Courier service

- One day before shipping, contact Client Services at Mayo 1-800-533-1710 and ask for Transportation. Alert transportation that a muscle biopsy will be shipped from your institution. Transportation will notify your courier to bring extra dry ice for packaging.
- Courier will pack the frozen specimen in 8-10 lbs of pellet or small particle dry ice and will prominently indicate that the package contains frozen biological material

If shipping via FedEx or Airborne

- Pack specimen in 8-10 lbs of pellet or small particle dry ice so the specimen is surrounded by the dry ice.
- Send to Mayo Medical Laboratories via an express overnight delivery service
- Shipping address:
  Mayo Medical Laboratories
  Attn: Muscle Sample Enclosed
  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 performed, contact:
  Andrew G. Engel, M.D. Mayo Clinic
  Phone: 507-284-5100
- To order Muscle Biopsy Shipping Kits (Supply T541), Phone 1-800-533-1710

Recommended methods for flash-freezing:

METHOD 1: SLURRY METHOD

- Place specimen vial in -70°C. freezer or on dry ice to pre-chill.
- 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 "snow cone."
- 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 at -70°C or on dry ice 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

- Place specimen vial in -70°C. freezer or on dry ice to pre-chill.
- 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. 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 beaker around so that the specimen will continue to contact the colder, frozen isopentane.
- The total freezing period should take approximately 10–15 seconds.
- After removing the specimen from the isopentane, immediately place the tissue in the container that has been pre-chilled on dry ice.
- Place cover on container. Puncture lid to allow excess isopentane to evaporate.
- **Keep specimen frozen at -70°C or on dry ice 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.