

Mitochondrial Diagnostic Laboratory Instructions for Collecting, Handling & Shipping Specimens

Address for Shipping, Mailing and Couriers:

Children's Hospital Colorado
Clinical Lab – Mitochondrial Diagnostic Laboratory
13123 East 16th Ave, B120 – Rm. B0200
Aurora CO 80045

All specimens must be received with an appropriate test requisition form that has been fully completed to include the following information:

- Name of patient
- Date of birth of patient
- Collection date and time for specimen(s)
- Name of physician ordering the test
- Name, address and contact phone number of facility requesting the test
- Your facility accession number for each specimen (for your reference)
- Test(s) requested – one form may be used for requesting multiple tests

Fibroblast for Respiratory Chain Enzyme Assays, BNPage with In-Gel Activity Staining Assay, and Pyruvate Dehydrogenase Enzyme Assay:

Please send two T-25 flasks containing confluent fibroblasts (with plug-seal caps, completely filled with media) in a Styrofoam box at room temperature.

Fibroblasts must be mycoplasma free and grown only in fetal calf serum of U.S. origin. Please provide documentation of mycoplasma testing results.

Mitochondrial Respiratory Chain Enzyme Assays (muscle, liver, and heart)

Specimen requirements:

- Muscle tissue: Please see the detailed instructions below. Minimum 60 mg required.
- Liver tissue: Please see the detailed instructions below. Minimum 20 mg required. Samples of <60 mg will require microassay (please note that the microassay is less robust than the regular assay).
- Heart tissue: Please see the detailed instructions below. Minimum 20 mg required.

MUSCLE

Muscle biopsies must be obtained without the use of electrocautery and must be completely processed within 5 to 10 minutes of being excised. Muscle biopsies should be preferably about 120 mg in size. They must be frozen immediately in liquid nitrogen or on dry ice. They cannot be placed in any preservative including OCT. They must be stored in a microvial at -70° C until shipment to the laboratory on dry ice.

LIVER

Liver samples can be obtained as a wedge biopsy or as a needle biopsy. It is important to ensure that sufficient liver tissue is obtained. If using a needle biopsy, obtain two (2) needle biopsies 14 Gauge Bard monopty instrument 2 cm length. Place the liver biopsy in either Parafilm or aluminum foil, then place in a 2 ml cryotube, and close the screw cap tightly. Freeze immediately in liquid nitrogen or on dry ice, and maintain in frozen condition. If possible, weigh

liver biopsy before placing in the cryotube. If the specimen was not weighed before it was frozen, DO NOT attempt to weigh a frozen sample as the samples degrade very easily. Instead, ship the sample to the Mitochondrial Diagnostic Laboratory without a weight. Lab personnel will weigh the sample.

HEART

Heart biopsies must be obtained without the use of electrocautery and must be completely processed within 5 to 10 minutes of being excised. Heart biopsies should be preferably about 20 mg in size (or more). They must be frozen immediately in liquid nitrogen or on dry ice. They cannot be placed in any preservative including OCT. They must be stored in a microvial at -70° C until shipment to the laboratory on dry ice.

Keep sample in -70° C freezer. Samples will not be stable at -20° C. Samples that have thawed even for a short period are not acceptable. Weighing the sample will cause thawing. DO NOT attempt to weigh a frozen sample.

Postmortem samples have to be obtained within 2 hours of death, but as soon as possible as interpretation will become more difficult with time.

BNPage with In-Gel Activity Staining (muscle, liver, and heart)

Instructions for obtaining samples are the same as detailed for Respiratory Chain Enzyme Analysis.

Specimen Requirements:

- Muscle: 100 mg required. If ordering both Respiratory Chain Enzyme Assays and BNPage Analysis, minimum requirement is 150 mg.
- Liver: 50 mg required. If ordering both Respiratory Chain Enzyme Assays and BNPage Analysis, minimum requirement is 80 to 120 mg.
- Heart: 50 mg required. If ordering both Respiratory Chain Enzyme Assays and BNPage Analysis, minimum requirement is 50 to 100 mg.

Samples smaller than minimal size may result in the lab not being able to process the sample or in reduced accuracy and reliability of the assay.

FOR ALL MITOCHONDRIAL ASSAY SPECIMENS:

Please enclose both the completed requisition and clinical information forms for mitochondrial. Direct contact information for the requesting physician is of importance to allow direct notification as needed. Incomplete paperwork can result in delayed processing.

Primary contact for mitochondrial enzyme assays:

Marisa Friederich, PhD

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Email: Marisa.Friederich@ChildrensColorado.org