Mitochondrial Enzyme Analysis

Aim-

This panel is designed to assess individual enzymatic activities of Complex I, II, III, and IV, and to assess the level of marker mitochondrial enzyme- Citrate Synthase. Selective or multiple deficiencies of these enzymes may permit the diagnosis of a mitochondrial myopathy.

Specimen requirements-

Freshly frozen muscle samples, maintained at -80°C after freezing, minimum required amount-50 mg.

Conditions for rejection-

To ensure the quality of the tests , muscle biopsies will be considered unacceptable under the following circumstances-

. If muscle is shipped frozen, but arrives thawed.
. The shipping box is damaged and the sample is destroyed.
. The muscle biopsy is under the required amount of 50 mg.
. The sample is not properly labeled, and accompanying paperwork is incomplete.

References-

1. DiMauro, S et al- Mitochondrial Myopathies, Ann Neuol 17:521-538,1985
2. DiMauro ,S et al- Mitochondrial Disorders in Neurology. Oxford Press,91-115, 1994.
3. DiMauro ,S et al- Neurology 30: 795-804, 1980.
4. Sottocasa, G.L. et al- J. Cell Biol 32:415-439, 1967.
5. Wharton, D.C. et al-Methods Enzymology 10:245-250, 1967.
6. Srere, PA – Methods Enzymology 13:3-11, 1969.
7. King, TE et al-Methods Enzymology 10:322-331, 1967.
8. King, TE et al-Methods Enzymology 10:275-294, 1967.
9. Lowry et.al – Am. J. of Physiology.244(Cell Physiology 13):C276-C287

Tissue Preparation-

Make a 1:10 homogenate in the following medium

Final conc.	Stock conc.	Vol/ 25 ml
50mM Tris HCl, pH 7.4	1M	1.25
0.15M KCl	3M	1.25

Use a glass homogenizer. Freeze the homogenate in Eppendorf tubes at -80°C for assays to be performed later.
Do the SDH and reductases first, then COX, Citrate Synthase last.

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