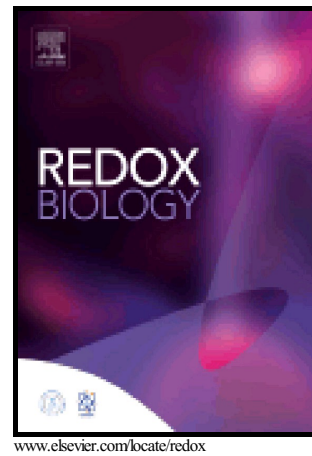


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Respiratory Analysis of Coupled Mitochondria in Cryopreserved Liver Biopsies

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Abstract

The aim of this work was to develop a cryopreservation method of small liver biopsies for *in situ* mitochondrial function assessment. Herein we describe a detailed protocol for tissue collection, cryopreservation, high-resolution respirometry using complex I and II substrates, calculation and interpretation of respiratory parameters. Liver biopsies from cow and rat were sequentially frozen in a medium containing dimethylsulfoxide as cryoprotectant and stored for up to 3 months at -80°C. Oxygen consumption rate studies of fresh and cryopreserved samples revealed that most respiratory parameters remained unchanged. Additionally, outer mitochondrial membrane integrity was assessed adding cytochrome *c*, proving that our cryopreservation method does not harm mitochondrial structure. In sum, we present a reliable way to cryopreserve small liver biopsies without affecting mitochondrial function. Our protocol will enable the transport and storage of samples, extending and facilitating mitochondrial function analysis of liver biopsies.

Abbreviations

ADP, adenosine diphosphate; **ATP**, adenosine triphosphate; **Ant A**, antimycin A; **Bak** BCL-2 antagonist killer 1; **cyt c**, cytochrome *c*; **DMSO**, dimethylsulfoxide; **EGTA**, ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid; **FCCP**, carbonyl cyanide-4-

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