

Video Article

# Respirometric oxidative phosphorylation assessment in saponin-permeabilized cardiac fibers

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## Abstract

Investigation of mitochondrial function represents an important parameter of cardiac physiology as mitochondria are involved in energy metabolism, oxidative stress, apoptosis, aging, mitochondrial encephalomyopathies and drug toxicity. Given this, technologies to measure cardiac mitochondrial function are in demand. One technique that employs an integrative approach to measure mitochondrial function is respirometric oxidative phosphorylation (OXPHOS) analysis. The principle of respirometric OXPHOS assessment is centered around measuring oxygen concentration utilizing a Clark electrode. As the permeabilized fiber bundle consumes oxygen, oxygen concentration in the closed chamber declines. Using selected substrate-inhibitor-uncoupler titration protocols, electrons are provided to specific sites of the electron transport chain, allowing evaluation of mitochondrial function. Prior to respirometric analysis of mitochondrial function, mechanical and chemical preparatory techniques are utilized to permeabilize the sarcolemma of muscle fibers. Chemical permeabilization employs saponin to selectively perforate the cell membrane while maintaining cellular architecture. This paper thoroughly describes the steps involved in preparing saponin-skinned cardiac fibers for oxygen consumption measurements to evaluate mitochondrial oxidative phosphorylation. Additionally, troubleshooting advice as well as specific substrates, inhibitors and uncouplers that may be used to determine mitochondria function at specific sites of the electron transport chain are provided. Importantly, the described protocol may be easily applied to cardiac and skeletal tissue of various animal models and human samples.

## Disclosures

No conflicts of interest declared.