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# Preparation of sub-mitochondria particles

We use sub-mitochondria particles (SMP) to study enzymatic activities of respiratory complexes. The conventional way to prepare mitochondrial particles with controlled orientation of their membrane is ultrasonication. Sonication disrupts the mitochondria membranes, which are in aqua environment seal back forming smaller vesicles. The orientation of proteins depends on the presence of $Mg^{2+}$ ions [1]. In the presence of $Mg^{2+}$ the mitochondria membrane resumes the right orientation with proteins facing inside the vesicles. In the absence of $Mg^{2+}$, the proteins face outside the vesicles. The idea of using the inside-out SMP is to expose the respiratory proteins to extra vesicular environment. The protocol was adapted from Panov [2] with modifications.

A sample of freshly isolated or thawed frozen mitochondria suspended in MiR05 respiration buffer is diluted with sonication buffer containing: 0.25 M sucrose, 10mM MOPS, 2 mM EDTA pH 8.0 in a final protein concentration 20-30 mg/ml.

1. Transfer 0.5 ml mitochondria suspension into Eppendorf. Place Eppendorf in a beaker with ice mixed with KCl (salt helps to decrease the temperature). During pulses immerse the warmed tip (6 mm) of the Q500 sonicator (Laboratory Supply Network, Inc, Atkinson, NH) into this mixture to cool it down, wipe tip and repeat sonication. The stable cooling environment is critical to avoid damage of membrane and proteins.

   Sonication program: amplitude 20%, four pulses by 5 sec.
2. Dilute the sonicated mitochondria with double volume of sucrose buffer and centrifuge at 16000g for 10 min to remove large mitochondria debris.
3. Collect supernatant for enzymatic assay.

## References

1. Lee C.P., Ernster L. Energy coupling in nonphosphorylating submitochondrial particles. In: Methods in Enzymology. 1967, 543-548.
2. Panov A. Practical Mitochondriology. Pitfalls and problems in studies of mitochondria. ISBN-10: 1483963853