**Mitochondrial complex activity assays**

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**ABSTRACT**

Mitochondria complex activity assays measure the activity levels of the different complexes of the mitochondrial electron transport chain (ETC).

**Protocol**

Mitochondria were isolated from HEK cells, iPSC-derived neurons, or midbrain organoids using the Qproteome Mitochondrial isolation kit as described above. Complex I (NADH oxidase/coenzyme Q reductase) was measured using the MitoCheck Complex I Activity Assay kit (Cayman Chemical, cat# 700930). The rate of NADH oxidation, which is proportional to CI activity, was determined by a decrease in absorbance at 340 nm over 15 min in the presence of ubiquinone and potassium cyanide to inhibit complex IV and prevent oxidation of ubiquinone. To assess CI, CII, and CIV function, we used a respirometry approach based on XFp Extracellular Flux Analysis 63. To this end, 3 mg of purified fresh mitochondria were resuspended in 200 μl of MAS buffer (70 mM sucrose, 220 mM mannitol, 5 mM KH2PO4, 5 mM MgCl2, 1 mM EGTA, 2 mM HEPES pH 7,4) and seeded in XFpSeahorse microplates. The plate was centrifugated at 2000 g for 5 min at 4°C. The OCR was measured before and after the serial addition of pyruvate + malate (5 mM each) + ADP 3,5 mM or 1mM Succinate + 4 µM rotenone, 4 µM rotenone + 8 µM antimycin A, 0,5 mM TMPD (N,N,N′,N′-tetramethyl-p-phenylenediamine dihydrochloride, Santa Cruz Biotechnology) + 1mM ascorbic acid, and 50 mM azide. Following each injection, three measurements for a total period of 15 min were recorded. Complex I-, II-, and IV-dependent respiration was calculated by subtracting OCR values from the substrates (Pyruvate + malate + ADP for CI, Succinate + rotenone for CII and TMPD + ascorbic acid for CIV) subtracted from the ones from the inhibitors (rotenone for CI, antimycin A + rotenone for CII and azide for CIV). The experimental values were normalized to the protein content per well via a BCA assay.