**Supplemental Material**

|  |  |
| --- | --- |
| **Chemical** | **Final concentration (mM)** |
| EGTA | 0.5 |
| MgCl2 | 3 |
| K-Lactobionate | 60 |
| Taurine | 20 |
| KH2PO4 | 10 |
| Sucrose | 110 |
| Bovine serum albumin | 1 mg/mL |
| HEPES | 20 (pH 7.0 at 37°$°$C) |

Table 1. Mitochondrial respiration media

Table 2. Mitochondrial respiration assay protocol

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagents added**  | **Final concentration in Oxygraph chamber (mM)** | **Action of reagent** | **Measurement Output** |
| Step 1* Glutamate
* Malate
* Pyruvate
 | * 10
* 5
* 10
 | CI substrates | CI leak respiration (CILeak) |
| Step 2* ADP
 | * 1.25
 | Substrate for ATP generation | CI oxidative phosphorylation |
| Step 3* Succinate
 | * 10
 | CII substrate | CI+CII oxidative phosphorylation1 |
| Step 4* Rotenone
 | * 0.001
 | CI inhibitor | Isolate flux to CII [CII(rot)] |
| Step 5* Oligomycin
 | * 0.0025
 | ATP-Synthase inhibitor | CI+CII leak respiration (CI,IILeak) |
| Step 6* FCCP
 | * 0.0015
 | Mitochondrial uncoupler | ETS capacity |
| Step 7* Antimycin A
 | * 0.005
 | CIII inhibitor | Residual oxidase consumption |

ADP, Adenosine diphosphate; ATP, Adenosine triphosphate; CI, Complex I; CI-OXPHOS, Complex I oxidative phosphorylation; CI+II-OXPHOS, Complex I + Complex II oxidative phosphorylation; CI,IILeak, Complex I + Complex II leak respiration; CILeak, Complex I leak respiration; CII, Complex II; CIII, Complex III; ETS, Electron transfer system; FCCP, Carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone; Rot, Rotenone

1The individual contribution of CII to oxidative phosphorylation (CII-OXPHOS) can also be derived (CI+II-OXPHOS minus CI-OXPHOS).