Assay of Complex I (NADH ubiquinone oxido-reductase)

Principle:

- Complex I in the respiratory chain transfers electrons from NADH to ubiquinone. Its activity will be assessed by following the decrease of NADH absorbance at 340 nm. Ubiquinone (or coenzyme Q_{10}), the very hydrophobic natural acceptor, is replaced by decylubiquinone, a more hydrophilic component.
- NADH is also oxidized by the activity of the NADH cytochrome b_5 oxido-reductase. This activity is insensitive to rotenone and is therefore evaluated by a parallel assay in the presence of rotenone, a specific inhibitor of the respiratory complex I.
- The specific complex I activity is the rotenone sensitive activity. It is calculated by subtracting the rotenone insensitive activity from the total NADH ubiquinone oxido-reductase activity.

Practical set up:

- 6 samples may be analyzed on the same day (5 patients and 1 control). This means 12 assays.
- Reaction medium composition:

  100 µM NADH
  100 µM decylubiquinone
  50 mM K Phosphate pH 7.5
  3.75 mg/mL BSA
  Tissue: 40 µg of proteins (post-nuclear supernatant from liver or muscle) or 4 µg of mitochondrial proteins
  Inhibition is performed with 12.5 µM rotenone.
- Preparation of reaction medium:

  1) In a 50 mL tube, prepare enough reaction medium for 7 samples i.e. 14 assays:

Reagents	Global quantity	Quantity/sample
500 mM K Phosphate pH 7.5	1540 µL	220 µL
50 mg/mL BSA	1155 µL	165 µL
25 mM decylubiquinone	63 µL	9 µL
H_2O	11564 µL	1652 µL

  2) In a 2 mL cuvette, add **2045 µL of the reaction medium and 44 µL of post nuclear supernatant (diluted at a final protein concentration of 2 mg/mL) or of isolated beef heart mitochondria (diluted at 0.2 mg/mL i.e. 1/200)**. Mix.
  3) Transfer twice **950 µL** of that mix in a 1 mL cuvette.
  4) Add **5 µL of 2.5 mM rotenone in one** of the two 1 mL cuvettes, mix.
- Assay:

  1) Reading in the spectrophotometer, at 37°C, at wavelength 340 nm
  Initial calibration is performed on air.
  2) Incubate the cuvettes **at 37°C, during 5 min,** in the spectrophotometer
  3) Start the reaction by adding **50 µL of 2 mM NADH kept at room temperature**.
  4) Reading every 15 seconds during 3 minutes,
  The two cuvettes containing the same sample are read in parallel at the same time.
  Four cuvettes may be read at the same time.
  If the decrease appears too rapid giving a non-linear curve, re-do the assay with less tissue.
- Calculation:

  1) Complex I specific activity is calculated as nanomoles/min/mg.
  2) The extinction coefficient for NADH is **ε = 6.2**
  3) The correction factor is therefore 4032.3 for 40 µg of proteins in the assay (tissue homogenate) and 40323 for 4 µg of proteins in the assay (isolated mitochondria)