Assay of Complex II (succinate ubiquinone oxido-reductase)

Principle:

- Complex II in the respiratory chain transfers electrons from succinate to ubiquinone. Its activity will be assessed by following the reduction of 2,6-dichlorophenolindophenol (DCPIP) by the decrease of the absorbance at 600 nm of the oxidized DCPIP. Ubiquinone (or coenzyme Q_{10}), the very hydrophobic natural acceptor, is replaced by decylubiquinone, a more hydrophilic component.
- Baseline is subtracted from the activity started by the addition of decylubiquinone to take into account any non-specific electron transfer to DCPIP.
- The specific complex II activity is sensitive to malonate. Sensitivty to malonate is usually above 90% of the activity observed without malonate. Activity in the presence of malonate is therefore not useful in routine diagnostic procedure.

Practical set up:

- 6 samples may be analyzed on the same day (5 patients and 1 control). This means 6 assays.
- Reaction medium composition:
  25 mM K Phosphate pH 7.5
  20 mM succinate
  100 µM decylubiquinone
  50 µM DCPIP
  1 mM KCN
  100 µM ATP
  2 mg/mL BSA
  Tissue: 40 µg of proteins (post-nuclear supernatant (liver or muscle) or cell suspension) or 4 µg of mitochondrial proteins
  Inhibition may be performed with malonate but is not mandatory for diagnosis.
- Preparation of reaction medium:
  1) In a 15 mL tube, prepare enough reaction medium for 7 samples i.e. 7 assays:

Reagents	Global quantity	Quantity/sample
500 mM K Phosphate pH 7,5	350 µL	50 µL
50 mg/mL BSA	280 µL	40 µL
200 mM succinate	700 µL	100 µL
5 mM DCPIP	70 µL	10 µL
10mM KCN	700 µL	100 µL
10mM ATP	70 µL	10 µL
H_2O	4662 µL	666 µL

  2) In a 1 mL cuvette, add **976 µL of the reaction medium and 20 µL of post nuclear supernatant or cell suspension** (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.
- Assay:
  1) Reading in the spectrophotometer, at 37°C, at wavelength 600 nm
  Initial calibration is performed on air.
  2) Incubate the cuvettes **at 37°C, during 5 min,** in the spectrophotometer
  3) Read baseline every 15 seconds during 3 minutes
  3) Start the reaction by adding **4 µL of 25 mM decylubiquinone kept at room temperature**.
  4) Reading every 15 seconds during 3 minutes,
  If the decrease appears too rapid giving a non-linear curve, re-do the assay with less tissue.
- Calculation:
  1) Complex II specific activity is calculated as nanomoles/min/mg proteins.
  2) The extinction coefficient for DCPIP is **ε = 19.2**
  3) The correction factor is therefore 1302.1 for 40 µg of proteins and 13021 for 4 µg of proteins in the assay.