Assay of Complex IV (cytochrome C oxidase)

Principle:

- Complex IV in the respiratory chain transfers electrons from reduced cytochrome c to oxygen. Its activity will be assessed by following the decrease of reduced cytochrome c absorbance at 550 nm.

Practical set up:

- 6 samples may be analyzed on the same day (5 patients and 1 control).
- Reaction medium composition:
  50 mM K phosphate pH 7.0 (25 mM for skin fibroblasts)
  100 µM reduced cytochrome c (50 µM for skin fibroblasts)
  Tissue: 40 µg of proteins (post-nuclear supernatant (liver or muscle) or cell suspension) or 1 µg of mitochondrial proteins
- Preparation of reaction medium:
  1) In a 15 mL tube, prepare 12 to 13 mL of 100 µM cytochrome c in 50 mM K phosphate pH 7.0
  2) Prepare a **"100% oxidized solution"** with 1mL of initial cytochrome c solution oxidized with few grains of **potassium ferricyanide** in a 1 mL cuvette (the color of the solution turns to a brownish dark red).
  3) Prepare a **"100% reduced solution"** with 1mL of initial cytochrome c solution reduced with few grains of **sodium dithionite** in a 1 mL cuvette (the color of the solution turns to salmon pink).
  4) Read the absorbance of the "100% oxidized solution" at 550 nm in the spectrophotometer after having obtained a blank on air. The absorbance should be around 0.7 for the 100 µM solution.
  5) Re-blank the spectrophotometer on the "100% oxidized solution" and read the absorbance of the "100% reduced solution". Its value is considered 100% reduction.
  6) Transfer an aliquot (50 or 100 µL) of the "100% reduced solution" in the initial cytochrome c solution and read its new absorbance. Keep on adding aliquots until reaching an absorbance between 90 and 95% of the 100% reduced solution absorbance. For example, if the 100% reduced solution had an absorbance of 1.4, the initial cytochrome c solution must be progressively reduced until its absorbance is between 1.26 and 1.33.
- Assay:
  1) Reading in the spectrophotometer, at 37°C, at wavelength 550 nm
  Initial calibration is performed on air.
  2) Incubate the cuvettes containing 980 µL of reduced initial solution of cytochrome c **at 37°C, during 5 min,** in the spectrophotometer
  3) Start the reaction by adding **20 µL of supernatant or cell suspension** (with proteins concentration set at 2 mg/mL) or of **beef heart mitochondria** (diluted at 0.05 mg/mL i.e. 1/800).
  4) Reading every 10 seconds during 3 minutes,
  Two 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 IV specific activity is calculated as nanomoles/min/mg of post-nuclear supernatant or cell suspension proteins.
  2) The extinction coefficient for cytochrome c is **ε = 18.5**
  3) The correction factor is therefore 1351.4 for 40 µg of proteins in the assay (tissue homogenate) and 54054 for 1 µg of proteins in the assay (isolated mitochondria)