# Assay of Complex III (ubiquinol cytochrome c oxido-reductase)

**Principle**:

- Complex III in the respiratory chain transfers electrons from ubiquinol (reduced form of ubiquinone (or coenzyme $Q_{10}$) to cytochrome c, the reduction of which is followed by the increase of absorbance at 550 nm.
- To evaluate complex III activity, two assays have to be performed concomitantly, one in the presence of antimycin A, specific inhibitor of the respiratory complex III. The specific complex III activity is the antimycin A sensitive activity. It is calculated by subtracting the antimycin insensitive activity from the total ubiquinol cytochrome c 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:
  200 µM decylubiquinol
  50 µM cytochrome c
  100 mM K Phosphate pH 7,5
  250 µM EDTA
  1 mM KCN
  Tissue: 30 µg of proteins (post-nuclear supernatant or cell suspension) or 0,75 µg of mitochondrial proteins
  Inhibition is performed with 12.5 µg/mL antimycin A.
- 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 | 3080 µL | 440 µL |
| 1 mM cytochrome c | 770 µL | 110 µL |
| 50 mM EDTA | 77 µL | 11 µL |
| 10 mM KCN | 1540 µL | 220 µL |
| $H_2O$ | 9576 µL | 1368 µL |

  2) In a 2 mL cuvette, add **2149 µL of the reaction medium and 33 µL of post nuclear supernatant** (diluted at a final protein concentration of 2 mg/mL) **or of isolated beef heart mitochondria** (diluted at 0.05 mg/mL i.e. 1/800). Mix.
  3) Transfer twice **992 µL** of that mix in a 1 mL cuvette.
  4) Add **5 µL of 2.5 mg/mL antimycin A** **in one** of the two 1 mL cuvettes, mix.
- Assay:
  1) Reading in the spectrophotometer, at 37°C, at wavelength 550 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 **8 µL of 25 mM decylubiquinol kept at 37°C**.
  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 increase appears too rapid giving a non-linear curve, re-do the assay with less tissue. The curve with isolated mitochondria is never linear, take into account the curve during the first minute.
- Calculation:
  1) Complex III specific activity is calculated as nanomoles/min/mg of post-nuclear supernatant proteins.
  2) The extinction coefficient for cytochrome c is **ε = 18.5**
  3) The correction factor is therefore 1801.8 for 30 µg of proteins and 72072 for 0.75 µg of proteins in the assay.