

Assay of combined complexes II + III (succinate cytochrome c oxido-reductase)

Principle:

- The combined activity of complexes II and III transfers the electrons from succinate to cytochrome c. This activity will be assessed by following the increase of absorbance of reduced cytochrome c at 550 nm.
- Subsequent oxidation of the reduced cytochrome c is inhibited by the cyanide added in the reaction medium.

Practical set up:

- 6 samples may be analyzed on the same day (5 patients and 1 control).
- Reaction medium composition:
 - 20 mM succinate
 - 100 μ M cytochrome c
 - 20 mM K Phosphate pH 7.5
 - 2.0 mg/mL BSA
 - 1 mM KCN
 - 100 μ M ATP
 - Tissue: 40 μ g of proteins (post-nuclear supernatant (liver or muscle) or cell suspension) or 4 μ g of mitochondrial proteins

- Preparation of reaction medium:

1) In a 15 mL tube, prepare enough reaction medium for 7 samples:

Reagents	Global quantity	Quantity/sample
500 mM K Phosphate pH 7.5	280 μ L	40 μ L
50 mg/mL BSA	280 μ L	40 μ L
200 mM succinate	700 μ L	100 μ L
10 mM KCN	700 μ L	100 μ L
10 mM ATP	70 μ L	10 μ L
H ₂ O	4200 μ L	1232 μ L

2) In a 2 mL cuvette, add **880 μ L of the reaction medium and 20 μ L 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.

- 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 **100 μ L of 1 mM cytochrome c kept at room temperature**.
- 4) Reading every 20 seconds during 3 minutes,
Six 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.

- Calculation:

- 1) Combined activity of complexes II + III is calculated as nanomoles/min/mg proteins.
- 2) The extinction coefficient for reduced cytochrome c is $\epsilon = 18.5$
- 3) The correction factor is therefore 1351.4 for 40 μ g of proteins in the assay and 13514 for 4 μ g of proteins in the assay.