Assay of citrate synthase

Principle:

- Citrate synthase is an enzyme of the tricarboxylic acid cycle (Krebs cycle). It catalyses the formation of citrate from oxaloacetate and acetylCoA. Citrate synthase activity is often used to evaluate the mitochondrial mass in a given tissue. The reduced CoA (CoA-SH) formed during the reaction transforms the 5,5' dithiobis 2 nitrobenzoic acid (DTNB) into 2-nitro-5-benzoic acid (TNB), which absorbs specifically at 412 nm. Citrate synthase activity will therefore be assessed by following the increase of TNB absorbance at 412 nm.
- A baseline activity exists and must be subtracted from the specific activity.

Practical set up:

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

  100 µM DTNB
  100 mM Tris HCl pH 8.1
  300 µM acetylCoA
  500 µM oxaloacetate
  0.1 % Triton X100
  Tissue: 40 µg of proteins (post-nuclear supernatant or cell suspension) or 4 µg of mitochondrial proteins
- 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
5 mM DTNB	140 µL	20 µL
10 mM acetylCoA	210 µL	30 µL
10% Triton X-100	70 µL	10 µL
1 M Tris HCl pH 8.1	665 µL	95 µL
H_2O	5425 µL	775 µL

  2)) In a 1 mL cuvette, add **930 µL of the reaction medium and 20 µL of post nuclear supernatant or skin fibroblasts (diluted at a final protein concentration of 2 mg/mL) or of isolated beef heart mitochondria diluted at 1/200**. Mix.
- Assay:

  1) Reading in the spectrophotometer, at 37°C, at wavelength 412 nm
  Initial calibration is performed on air.
  2) Incubate the cuvettes **at 37°C, during 5 min,** in the spectrophotometer
  3) Read baseline every 20 seconds during 4 minutes
  Six cuvettes may be read at the same time.
  4) Start the reaction with **50 µL of 10 mM oxaloacetate in 100 mM Tris HCl pH 8.1**
  5) Read the reaction every 20 seconds during 4 minutes
  Six cuvettes may be read at the same time.
  If the increase of absorbance appears too rapid giving a non-linear curve, re-do the assay with less tissue.
- Calculation:

  1) Citrate synthase activity is calculated as nanomoles/min/mg proteins.
  2) The extinction coefficient for DTNB is **ε = 13.6**
  3) The correction factor is therefore 1838.2 for 40 µg of proteins and 18382 for 4 µg of proteins in the assay.