Conditions used in the consensus protocols for the spectrophotometric assays of respiratory chain activities

	I	II	III	IV	I+III	II+III	CS
pH	7.5	7.5	7.5	7.0	7.5	7.5	8.0
Phosphate	50	25	100	50 (25 for fibros)	50	20	-
Tris	-	-	-	-	-	-	100
Inhibitor	12.5 µM rot	1 mM KCN	12.5 µg/mL antim 1 mM KCN	-	1 mM KCN 12.5 µM rot	1 mM KCN	-
Substrates	100 µM NADH 100 µM dUb	20 mM succ. 100 µM dUb 50 µM DCPIP	200 µM dUb-H_2 50 µM cytC	100 µM cytC-H_2 (50 for fibros)	200 µM NADH 100 µM cytC	20 mM succ. 100 µM cytC	100 µM DTNB 300 µM ACoA 500 µM OA
Adjuvants	3.75 mg/mL BSA	2 mg/mL BSA 100 µM ATP	250 µM EDTA	-	1 mg/mL BSA	100 µM ATP 2 mg/mL BSA	0.1% Triton-X100
λ (nm)	340	600	550	550	550	550	412
Subtraction	± rot	Baseline	± antim	-	±rot	-	Baseline
Start	NADH	dUb	dUb-H_2	Tissue	NADH	cytC	OA
Reading	3	3	3*	3	3	3	4
Tissue	40 (4)	40 (4)	30 (0.75)	40 (1)	40 (4)	40 (4)	40 (4)
Calculation factor	4032.3 (40323)	1308.9 (13089)	1801.8 (72072)	1351,4 (54054)	1351.4 (13514)	1351.4 (13514)	1838.2 (18382)

Phosphate**:** concentration of potassium phosphate buffer expressed as millimoles/L; fibros=fibroblasts; Tris: concentration of Tris buffer expressed as mmoles/L; Inhibitor: rot= rotenone, KCN=potassium cyanide, antim= antimycin A. Substrates: dUb=decylubiquinone, succ.= succinate, DCPIP=2,6-dichlorophenolindophenol, dUb-H_2= decylubiquinol, cytC= cytochrome C, cytC-H_2= reduced cytochrome C, ACoA= acetylCoenzyme A, OA= oxaloacetate, DTNB=5,5' dithiobis 2 nitrobenzoic acid; Adjuvants: reagents added to the assay medium that are not substrates nor inhibitors: BSA=bovine serum albumin; Subtraction: ±rot or ±antim= the activity is measured in two parallel cuvettes, with and without the specified inhibitor. The specific activity of the respiratory chain is inhibitor sensitive and is calculated by subtracting the activity measured in the presence of the inhibitor from that measured in its absence, baseline=the specific activity is calculated by subtracting the activity in the absence of the specific substrate from the activity observed after its addition; λ: wavelength, the extinction coefficient used were 6.2 for NADH at 340 nm, 19.1 for DCPIP at 600 nm, 18.5 for cyt C at 550 nm, and 13.6 for TNB (2-nitro-5-benzoic acid) at 412 nm; Start: compound used to start the reaction; reading: duration of the reaction measurement expressed as minutes; Tissue: amount of tissue used for the assay expressed as micrograms per assay, the numbers between brackets are the amount of isolated beef heart mitochondria and the calculation factor appropriate for that tissue; * reading is performed during the first minute only for beef heart mitochondria.