

## Reagents for spectrophotometric assays of respiratory chain activities

### ASSAYS OF 5 PATIENTS' SAMPLES + ONE CONTRÔL

#### REAGENTS TO BE PREPARED EXTEMPORANEOUSLY

Reagents	Reconstitution	Storage
100 $\mu$ M reduced cytochrome c (Sigma C-7752 ; FW 12384, corrected 12900)	Weigh at least 15 mg cytochrome c, dilute in 50 mM K phosphate pH 7.0 at 0.930 mL/mg then reduce according to the complex IV assay protocol	-20°C
1 mM cytochrome c (Sigma C-7752 ; FW 12384, corrected 12900)	Weigh at least 20 mg cytochrome c (at least 30 mg if combined I+III activity is measured), dilute in distilled water at 75.3 $\mu$ L/mg	-20°C
10 mM oxaloacetate (Sigma O-4126 ; FW 132,1 corrected 134,8)	Weigh at least 2 mg oxaloacetate, dilute at 0.758 mL/mg in 0,1 M Tris HCl pH 8.1	-20°C
2 mM NADH (Sigma N-8129 ; FW 709.4, corrected 786.8)	Weigh at least 2 mg NADH (at least 3 mg if combined I+III activity is measured), dilute at 0.685 mL/mg in distilled water	0-6 °C
100% dithionite (sodium hydrosulfite) (Sigma S-1256 ; FW 174.1, corrected 197.8)	Weigh at least 200 mg dithionite, dilute at 1 $\mu$ L/mg in distilled water, put in boiling water bath till complete dissolution, immediately use to reduce decylubiquinone.	Dessicator, Room temperature
21.3 mM decylubiquinol (Sigma D-7911 ; FW 322.4 corrected 329.0)	To 176 $\mu$ L 25 mM decylubiquinone kept as aliquot at -20°C, add 30 $\mu$ L of 100% dithionite solution (see above), vortex, incubate at 37°C in distilled water bath (the solution must turn white within 15 -20 min)	-20°C
10 mM KCN (Fluka 60179 ; FW 65.12)	Weigh a grain of KCN, dilute at 1.536 mL/mg in distilled water	In special cupboard for toxic compounds, Room temperature

**REAGENTS KEPT AT -20°C**

<b>Reagents</b>	<b>Reconstitution</b>	<b>Powder storage</b>
5 mM DTNB (Sigma D-8130; FW 396.3)	Weigh DTNB, dilute at 0.505 mL/mg in 95% ethanol, 500 $\mu$ L aliquots	Dessicator, Room temperature
10 mM acetylCoA (Sigma A-2181; FW 827.4 corrected 891.4)	Weigh acetylCoA, dilute at 0.112 mL/mg in distilled water	-20°C
25 mM decylubiquinone (Sigma D-7911; FW 322.4 corrected 329.0)	Add 1.216 mL DMSO in a 100 mg decylubuquinone vial to obtain a 250 mM solution, dilute to 25 mM decylubuquinone with DMSO, prepare 88 and 176 $\mu$ L aliquots.	-20°C
200 mM succinate (Sigma S-7501; FW 118.1)	Weigh 472.4 mg, dilute in 15 mL water, adjust pH to 7.4 with KOH, complete the volume to 20 mL with distilled water, prepare 500 $\mu$ L aliquots.	Dessicator, Room temperature
50 mg/mL Fatty acid free bovine serum albumin (Sigma A-2153)	Weigh albumin, dilute at 20 $\mu$ L/mg in distilled water, prepare 1 mL aliquots	0-6°C
2.5 mg/mL antimycin A (Sigma A-8674)	Weigh antimycin A, dilute at 0.4 mL/mg in 95% ethanol.	-20°C
2.5 mM rotenone (Aldrich R200-1; FW 394.4)	Weigh rotenone, dilute at 1.014mL/mg in 95% ethanol/DMSO (50/50), prepare 200 $\mu$ L aliquots.	-20°C

## REAGENTS KEPT AT 0-6°C

Reagents	Reconstitution	Powder storage
5 mM DCPIP (sodium 2,6-dichlorophenol-indophenol (Sigma D-1878; FW 290.1, corrected 326.1)	Weigh 72.5 mg DCPIP, dilute with 50 mL water, solution stable only one month.	Room temperature, Dessicator
50 mM K phosphate pH 7.0 (potassium dihydrogenophosphate (K <sub>1</sub> ) Prolabo 33611-265 FW 136.09; Dipotassium hydrogenophosphate (K <sub>2</sub> ) Prolabo 33612-268 FW 174.18)	Weigh 3.4 g K <sub>1</sub> powder, dilute in 500 mL distilled water; weigh 4.35 g K <sub>2</sub> powder, dilute in 500 mL water; Mix the 2 solutions under pH control adding K <sub>1</sub> solution in K <sub>2</sub> solution up to pH 7.0; prepare 50 mL aliquots.	Room temperature
500 mM K phosphate pH 7.5 (potassium dihydrogenophosphate (K <sub>1</sub> ) Prolabo 33611-265 FW 136.09; Dipotassium hydrogenophosphate (K <sub>2</sub> ) Prolabo 33612-268 FW 174.18)	Weigh 34 g K <sub>1</sub> powder, dilute in 500 mL distilled water; weigh 43.5 g K <sub>2</sub> powder, dilute in 500 mL water; Mix the 2 solutions under pH control adding K <sub>1</sub> solution in K <sub>2</sub> solution up to pH 7.5; prepare 50 mL aliquots.	Room temperature
1 M Tris HCl pH 8.1 (Prolabo 28811-295 ; FW 121.14)	Weigh 6.06 g Tris, dilute in 30 mL water, adjust pH at 8.1 with 5N then 1N HCl, dilute up to 50 mL with distilled water	Room temperature
100 mM Tris HCl pH 8.1 (Prolabo 28811-295 ; FW 121.14)	Dilute 1 M solution to 0.1 M with distilled water	Room temperature
10% Triton-X100 (solution Sigma X-100)	Dilute 5 mL Triton-X100 solution with 45 mL water	Room temperature (solution 100%)
"Mannitol buffer" = pH 7.2 225 mM mannitol 75 mM sucrose 10 mM Tris HCl 0,1 mM EDTA (Mannitol Sigma M-9546; FW 182.2 - Saccharose Fluka 84105; FW 342.3 - EDTA Sigma E-7889; 0.5 M solution)	Weigh 2.05 g mannitol, 1.28 g sucrose, 60.6 mg Tris, add 10 µL 0.5 M EDTA solution, dilute with 40 mL water, adjust pH at 7.2 and dilute up to 50 mL with distilled water.	Room temperature