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Electron Transport Chain Protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes the procedure for analyzing Electron Transport Chain activity of Complexes 1, 2, and 4 in mitochondria isolated from fibroblasts used by the Seifert Lab. It follows the "Electron Transport Chain Protocol" (ETCP) described in:
Vekaria, H.J., Kalimon, O.J., Prajapati, P., Velmurugan, G.V., Sullivan, P.G., 2024. An efficient and high-throughput method for the evaluation of mitochondrial dysfunction in frozen brain samples after traumatic brain injury. Front Mol Biosci 11, 1378536.
which is a modified version of the "Respirometry in Frozen Samples" (RIFS) assay from:
Acin-Perez, R., Benador, I.Y., Petcherski, A., Veliova, M., Benavides, G.A., Lagarrigue, S., Caudal, A., Vergnes, L., Murphy, A.N., Karamanlidis, G., Tian, R., Reue, K., Wanagat, J., Sacks, H., Amati, F., Darley-Usmar, V.M., Liesa, M., Divakaruni, A.S., Stiles, L., Shirihai, O.S., 2020. A novel approach to measure mitochondrial respiration in frozen biological samples. Embo j 39, e104073.

1 Thaw frozen mitochondrial samples

2 Add 25 µL Mitochondria Isolation Buffer (MIB; [M] 215 millimolar (mM) mannitol, [M] 75 millimolar (mM) sucrose, 0.1% BSA, [M] 20 millimolar (mM) HEPES, and [M] 1 millimolar (mM) EGTA, adjusted to pH 7.2 with KOH)

3 Gently resuspend pellet

4 Measure concentration of 4 µL of each mitochondrial mix by BCA

5 Prepare

Equipment

Seahorse XFe96 Analyzer	NAME
Agilent	BRAND
BLE2100298	SKU
https://www.agilent.com/en/product/cell-analysis/real-time-cell-metabolic-analysis/xf-analyzers/seahorse-xfe96-analyzer-740879	LINK

96-well cartridge ports as follows:
Port A (ETC 1): 3220 µL Mitochondrial Respiration buffer w/o BSA, 2.8 µL Rotenone, 280 µL succinate;
Port B: 3500 µL Mitochondrial Respiration buffer w/o BSA, 3.5 µL Antimycin A;
Port C: 3325 µL Mitochondrial Respiration buffer w/o BSA, 88 µL Ascorbate, 88 µL TMPD;
Port D: 3033 µL Mitochondrial Respiration buffer w/o BSA, 480 µL Azide.

6 Load cartridge well ports with 25 µL of port mixes

7 On ice Load 75 µL of mitochondrial sample (2 µg protein) per well

8 Balance the plate and spin at 3214 rcf, 4°C, 00:10:00

9 Prepare 11 mL Alamethicin Assay solution (10.6 mL Mitochondrial Respiration buffer w/o
BSA, [M] 40 mg/mL Alamethicin 9.63 µL , [M] 1.5 millimolar (mM) NADH 385 µL , [M] 10 micromolar (µM) cytochrome c 48 µL)

10 Add 100 µL assay solution to each well

11 Run plate on

Equipment

Seahorse XFe96 Analyzer	NAME
Agilent	BRAND
BLE2100298	SKU
https://www.agilent.com/en/product/cell-analysis/real-time-cell-metabolic-analysis/xf-analyzers/seahorse-xfe96-analyzer-740879	LINK

sequentially injecting the contents of Ports A-D over 01:00:00

Protocol references

Vekaria, H.J., Kalimon, O.J., Prajapati, P., Velmurugan, G.V., Sullivan, P.G., 2024. An efficient and high-throughput method for the evaluation of mitochondrial dysfunction in frozen brain samples after traumatic brain injury. Front Mol Biosci 11, 1378536.