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Working

Measurement of cellular oxygen consumption and extracellular acidification

Version 2

PLOS One

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Sumi C, Matsuo Y, Kusunoki M, Shoji T, Uba T, Iwai T, Bono H, Hirota K (2019) Cancerous phenotypes associated with hypoxia-inducible factors are not influenced by the volatile anesthetic isoflurane in renal cell carcinoma. PLoS ONE 14(4): e0215072. doi: 10.1371/journal.pone.0215072

BEFORE STARTING

The cellular oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using an XFp Extracellular Flux Analyzer™ (Agilent Technologies, Santa Clara, CA).

1 Cells were seeded on to the XFp Cell Culture microplate and incubated overnight.

2 The sensor cartridge of the XFp Analyzer was hydrated at 37 °C in a non-CO_2 incubator one day before the experiment.

3 For the OCR assay, injection port A on the sensor cartridge was loaded with oligomycin (a complex V inhibitor, final concentration 1 µM), carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP; an uncoupling agent, final concentration 2 µM) was loaded to port B, and rotenone/antimycin A (inhibitors of complexes I and III, final concentration 0.5 µM each) was loaded to port C.

4 During sensor calibration, cells were incubated at 37 °C in the non-CO_2incubator in 180 µl assay medium (XF Base Medium: 25 mM glucose, 1 mM pyruvate, and 2 mM L-glutamine, pH 7.4).

5 The plate was immediately placed on the calibrated XFp Analyzer for the assay.