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Assessment of Mitochondrial OCR For SH-SY5Y Cell using Agilent Seahorse XFe Cell Mito Stress Test

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We use this protocol and it's working

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Abstract

This protocol describes the assessment of mitochondrial function in SH-SY5Y cells using the Agilent Seahorse XFe Cell Mito Stress test, which measures the oxygen consumption rate (OCR) as an indicator of mitochondrial function. Additionally, it evaluates the effects of iron supplementation and reactive oxygen species (ROS) inhibition.

Cell Seeding and Treatment

2d

1 Seed 80,000 cells (SH-SY5Y) in 180 µl media in each well of a Seahorse XFe96 microplate.
Note: Leave well A blank empty as background control.

1.1 For iron supplementation, treat cells with 0.2 mg/mL ferric ammonium citrate (FAC)
For ROS inhibition, treat cells with 250 nM Liproxstatin-1

1.2 Incubate the plate at 37 °C with 5% CO2 for 48:00:00 2d

Preparation of Sensor Cartridge

2 Hydrate the sensor cartridge

2.1 The day prior to measuring OCR, hydrate the sensor cartridge with Seahorse XF Calibrant 200 µL /well and 400 µL PBS/ edge well and keep the sensor cartridge at 37 °C in a non-CO2 incubator overnight

Preparing the Assay Medium

1h

3 On the day of the assay, replace the growth medium with pre-warmed assay medium (180 µL) containing Seahorse XF base medium, 1 mM pyruvate, and 20 mM glucose.

3.1 Incubate the microplate in a non-CO2 incubator at 37 °C for 01:00:00 to equilibrate the temperature and pH. 1h

Preparing Modulating Agents

4 In the meantime, prepare following modulating agents (10X) in assay medium for injection:

	A	B	C	D
	Port Loading Design	**10X concentration**	**Add to Port volume**	**Final well concentration (µM)**
	Port A: Oligomycin	10 µM	20 µL	1 µM
	Port B：2,4-Dinitrophenol (DNP)	500 µM	25 µL	50 µM
	Port C：Rotenone	5 µM	27 µL	0.5 µM

4.1 Load 10X concentration of agents into port of the cartridge (From step 2.1)

Calibrating the Analyzer

15m

5 Place the loaded sensor cartridge into analyzer and run the program. The calibration usually takes 00:15:00 .

15m

Measuring the OCR

6 After calibration is done, transfer the Seahorse XFe96 cell microplate (from step 3.1) to the analyzer and press "continue"

Post-Assay Procedures

7 Protein Normalization:

7.1 After the assay, aspirate the assay medium and lyse the cells with a suitable lysis buffer and measure protein concentration using a BCA assay.

7.2 Use this data for normalizing the OCR results.