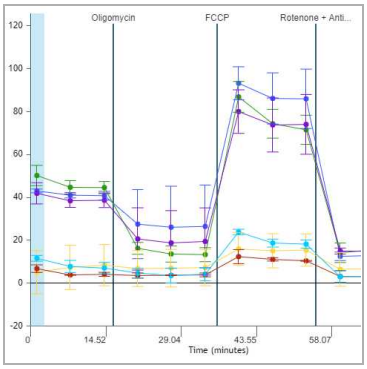


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# Assessment of oxidative phosphorylation and glycolysis in NK cells (Seahorse assays)

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## DISCLAIMER

These protocols are based upon the manufacturer's instructions and protocols provided by Ameeta Kelekar's lab (University of Minnesota).

## ABSTRACT

The rate of glycolysis (as measured by acidification of the extracellular space) and the rate of oxidative phosphorylation (as calculated from the consumption of oxygen) are measured in cells before and after the addition of drugs that test the resting and maximal capacity of these cells to respond.

Agilent offers a Mito Stress test kit (cat. 103015-100) to analyze both extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) in a combined assay that is the most commonly used in the literature. This assay is conducted in the presence of glucose. The glycolytic rate assay (cat. 103344-100) and ATP rate assays (cat. 103592-100) determine basal ECAR in the media that initially does not contain glucose and thus gives a more precise measure of glycolysis.

OPEN ACCESS

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

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MATERIALS

MATERIALS

- Seahorse XFp FluxPak **Agilent Technologies Catalog #103022-100**
- Sodium Pyruvate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5280**
- Seahorse XF Assay Medium **Agilent Technologies Catalog #103575-100**
- Poly-L-Lysine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P4707-50ML**
- Seahorse XF 1.0 M glucose solution **Agilent Technologies Catalog #103577-100**
- Seahorse XF Glycolytic Rate Assay Kit **Agilent Technologies Catalog #103344-100**
- Seahorse XF Real-Time ATP Rate Assay Kit **Agilent Technologies Catalog #103592-100**
- Seahorse XF Cell Mito Stress Test Kit **Agilent Technologies Catalog #103015-100**

Poly-L-lysine solution (Cat. No. P4707-50mL, MilliporeSigma)

## Day 0

1 Day before assay: Hydrate seahorse sensor cartridge

Add 200 µL of sterile water to each well of the Seahorse utility plate.

2 Add Seahorse Calibrant to a 50 mL conical tube. Place both the cartridge and aliquoted calibrant at 37°C in a non-$CO_2$ incubator overnight.

## Day 1

3 Day of assay:

Replace water in utility plate with 200 µL Seahorse calibrant per well and place back into the non-$CO_2$ incubator

4 Make sure that the Seahorse XF Assay medium (Agilent Technologies) is at pH 7.4

5 Wash NK cells twice, once with PBS and once with Seahorse XF Assay medium (Agilent Technologies)

6 NK cells are resuspended in Seahorse XF Assay Medium and added to a 96 well or 24 well flat bottom plate that has been coated with 0.01% poly-L-Lysine solution (Millipore Sigma) according to the manufacturer's instructions.

6.1 *Option 1*: 500,000 cells/well are added to a **96 well** plate.

6.2 *Option 2*: 1 million cells/well are added to a **24 well** plate.

7 Spin down cells to adhere to the plate. Spin plate at 40 g (500 RPM) for 4 minutes. Rotate plate 180° and then spin cells at 80 g (700 RPM) for 4 minutes without brake.

7.1 Check for even plating under microscope.

7.2 Put cell plate in the non-$CO_2$ incubator while preparing drugs.

8 Prepare drugs and add 25 µL into ports for a 96 well seahorse assay or 75 µL into ports for a 24

well seahorse assay

9 The extracellular acidification rate (ECAR) and the oxygen consumption rate (OCR) are measured (pmoles/min) in real time in an XFe96 analyzer after injection of drugs in the specified order.

*e.g.*

- *Mito Stress Test kit (10 mM glucose, 1 µM oligomycin then 1 µM FCCP plus 1mM sodium pyruvate then 0.5 µM rotenone/antimycin A; Cat. No. 103015-100)*
- *Glycolytic rate assay kit (0.5µM rotenone/antimycin A then 50mM 2-DG; Cat. No. 103344-100)*
- *ATP Rate Assay kit (1µM oligomycin then 0.5µM rotenone/antimycin A; Cat. No. 103592-100; all Agilent Technologies)*

10 The spare respiratory capacity (SRC) is calculated from the change from basal oxygen consumption, after addition of glucose, to maximal oxygen consumption, after addition of FCCP.