Glycolysis Stress Test for Monocyte Glycolytic Function

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dx.doi.org/10.17504/protocols.io.ufaetie

Oct 08, 2018

Working

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Cell-Tak	354240	Corning
Seahorse XFp FluxPak	103022-100	Agilent Technologies
Seahorse Base Medium DMEM	102353-100	Agilent Technologies
200 mM L-Glutamine	G7513	Sigma
0.1 M Sodium Hydroxide Solution	71395	Sigma
Seahorse XFp Glycolysis Stress Test	103017-100	Agilent Technologies
0.1M sodium bicarb buffer pH 8.0 sterile filtered	View	Contributed by users
Pipettes and P1000 P200 P10 tips	View	Contributed by users

Day Prior to Assay

1 Turn on XFp analyzer to warm up overnight

Hydrate XFp sensor cartridge
Add 200 µl XF calibration solution (included with FluxPak) to each well.
Add 400 µl sterile PBS or H_2O to each moat.
Incubate overnight at 37^{o}C in non-CO_2 incubator.

Materials to Prepare

2 **Seahorse DMEM Media**
Add 50 µl pyruvate, 50 µl L-glutamine, to 5 ml Seahorse Base Medium. Sterile filter before use.

Cell-Tak coated XFp plate
Prepare Cell-Tak. Add Cell-Tak and sodium hydroxide to 0.1 M sodium bicarbonate so that each well will receive 0.56 µg Cell-Tak, and sodium hydroxide concentration will be 0.63 mM. Cell-Tak concentration varies by batch, so calculations for each new batch will need to be performed. Add 25 µl Cell-Tak solution to each well. Plate can be stored at room temperature (at least 20 minutes) until use (for a few hours). Plates can also be prepped prior to assay day by incubating plates in Cell-Tak (at least 20 minutes), then aspirating remaining solution, air drying, and storing at 4^{o}C until use.

Monocytes
Monocytes should be prepared as directed in the monocyte isolation protocol. Isolated monocytes should be diluted to a concentration of 3×10^{6} cells / ml in prepared Seahorse DMEM media prior to use in the assay. Use monocytes immediately.

Procedure

3 Aspirate Cell-Tak solution if not already done.

4 Add 50 µl medium to wells A and H and 50 µl cells to wells B-G. Samples are generally run in duplicate or triplicate on each plate. Cell number is 1.5×10^5 cells per well.

5 Place plate in the carrier and place in centrifuge. Spin 300×g for 1 minute without brake.

6 Add 130 µl assay medium to each well A-H (final volume 180 µl).

7 Incubate plate at 37^{o}C in non-CO_2 incubator for 1 hour.

8 While plate is incubating, perform steps 9-13

9 Prepare preliminary drug dilutions (mix by pipetting up and down)
100 mM glucose (blue cap) - add 300 µl medium
50 µM oligomycin (light blue cap) - add 288 µl medium
500 mM 2-deoxyglucose (green cap) - add 300 µl medium - vortex 1 minute

10 Prepare final drug dilutions
100 mM glucose - use as-is
10 µM oligomycin - 120 µl of 50 µM oligomycin in 480 µl medium
500 mM 2-deoxyglucose - use as-is

11 Remove sensor cartridge from incubator and remove and reinsert sensors briefly to clear air bubbles.

12 Fill cartridge:
Port A (all wells): 20 µl glucose (10 mM final concentration)
Port B (all wells): 22 µl oligomycin (1 µM final concentration)
Port C (all wells): 25 µl oligomycin (2 µM final concentration)
Port D (all wells): 27 µl 2-deoxyglucose (50 mM final concentration)

> **NOTE**
> We do two separate injections of oligomycin, although one seems to work (although less consistently). If one injection is desired, prepare oligomycin so that final concentration after the first injection is 2 µM.

13 Select Glycolysis Stress Test program on Seahorse XFp and calibrate sensor cartridge (remove lid).

> **NOTE**

If two oligomycin injections are used, a custom program will need to be run.

14 After 1 hour cell incubation, remove utility plate from XFp and insert cell plate (remove lid).

15 Run Assay. 3 measurements per injection (including basal) is sufficient.

16 After run is completed, image each well by photomicroscopy or collect and isolate protein from each well to normalize cell numbers.

Data Analysis

17 Data can be analyzed in the following manner. Averages or min/max for each condition can be used.

Glycolysis: (glucose ECAR) - (2-deoxyglucose ECAR)
Glycolytic Capacity: (oligomycin ECAR) - (2-deoxyglucose ECAR)
Glycolytic Reserve: (oligomycin ECAR) - (glucose ECAR)

Calculations are depicted in Figure 1.

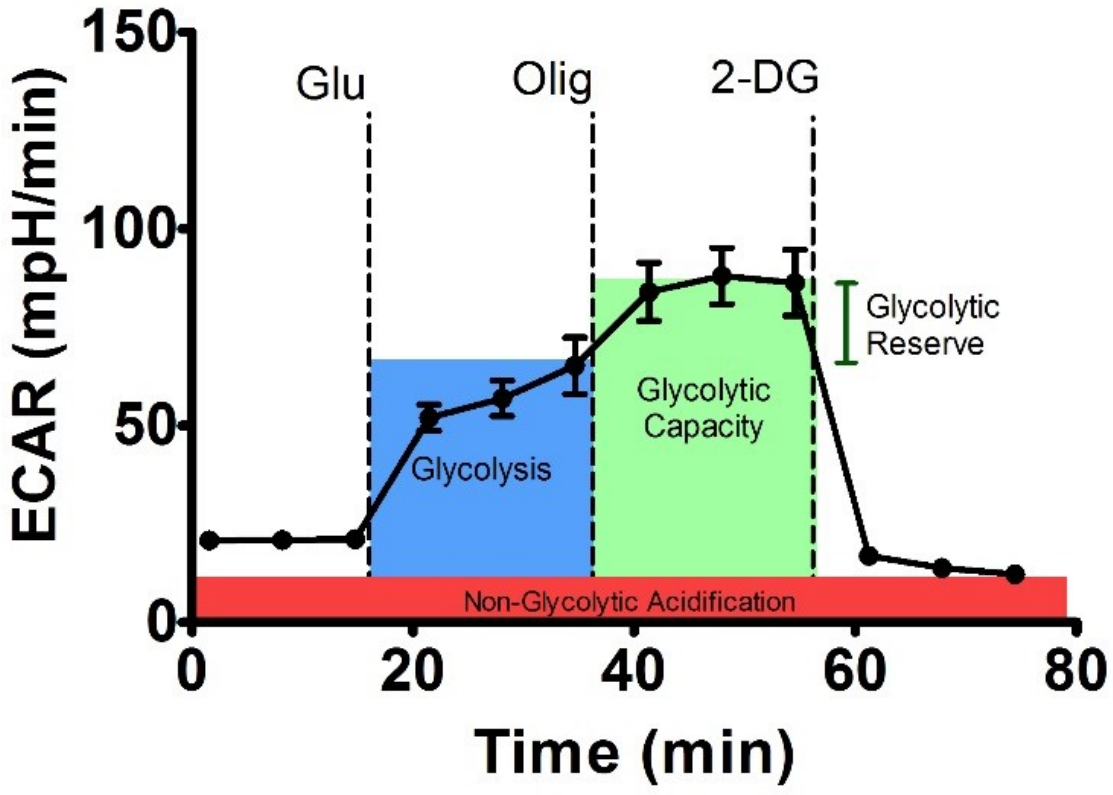

Figure 1. Calculations for Glycolysis Stress Test.Notes