Immediate Metabolic Response to LPS in Monocytes

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

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
100 mM Sodium Pyruvate	S8636	Sigma
200 mM L-Glutamine	G7513	Sigma
45% D-( )-Glucose	G8769	Sigma
0.1 M Sodium Hydroxide Solution	71395	Sigma
Lipopolysaccharide (1 microgram/ml in LPS stored -20decC)	L4391	Sigma Aldrich
0.1M Sodium Bicarbonate Buffer pH 8.0 Sterile	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, 20 µl glucose 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°C in non-CO_2 incubator for 1 hour.

8 While plate is incubating, perform steps 9-12.

9 Prepare LPS dilution
Thaw 1 µg/ml LPS stock
Dilute 30 µl LPS stock in 270 µl assay medium (100 ng/ml)

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

11 Fill cartridge:
Port A (wells A-D, H): 20 µl assay medium (**media control condition**)
Port A (wells E-G): 20 µl LPS dilution (10 ng/ml final concentration)

> **NOTE**
> The listed plate setup is for a comparison between LPS and media in triplicate. This can be varied depending on the research question. For example, we compare LPS responses +/- glucose in triplicate in this assay. We have also used media, LPS, and an inhibitor+LPS in duplicate in this assay.

12 Set up program on Seahorse XFp and calibrate sensor cartridge (**remove lid**).
Basal: 5 measurements
Injection 1: 23 measurements

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

14 Run Assay.

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

Data Analysis

16 Data can be analyzed in several ways. The magnitude of the ECAR response can be measured by **max ECAR - min ECAR** or simply by **max ECAR**. The overall response kinetics are best measured by **area under the curve** (trapezoidal method).

An example assay is shown in Figure 1.

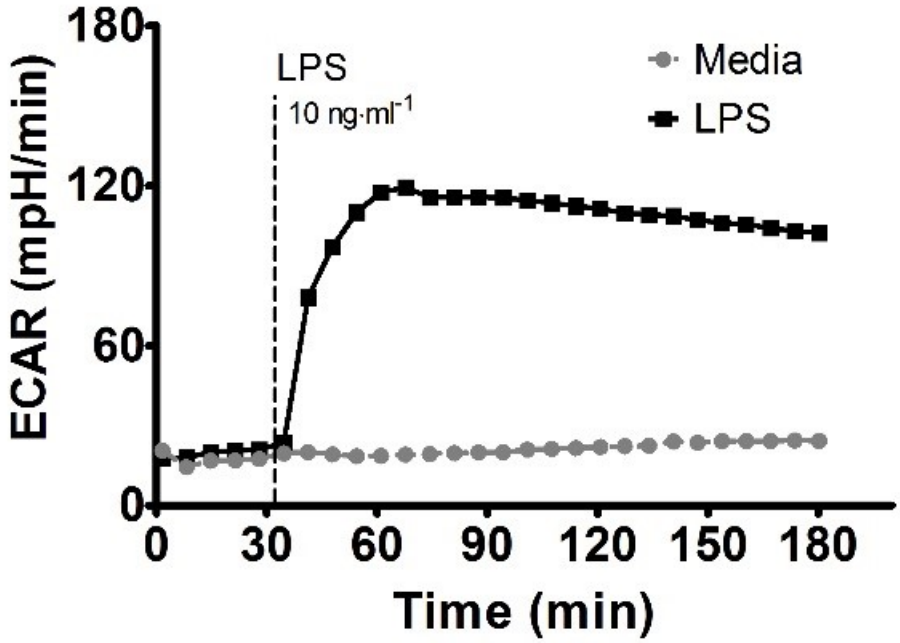

Figure 1. Example assay