24 Hour Metabolic Response to LPS in Monocytes

Brandt Pence[1]

[1]University of Memphis

dx.doi.org/10.17504/protocols.io.ufdeti6

Oct 08, 2018

Working

Brandt Pence
University of Memphis

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
RPMI-1640 without phenol red	11835055	Gibco - Thermo Fisher
Fetal Bovine Serum	10437028	Gibco - Thermo Fisher
Penicillin-Streptomycin	15140122	Gibco - Thermo Fisher
0.1M Sodium Bicarbonate Buffer pH 8.0 Sterile	View	Contributed by users
Pipettes and P1000 P200 P10 tips	View	Contributed by users

Day 1 Materials to Prepare

1 **Stimulation media**

Add 500 µl fetal bovine serum and 50 µl pen-strep to 4.45 ml RPMI, sterile filter

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 $3{\times}10^{6}$ cells / ml in prepared stimulation media prior to use in the assay. Use monocytes immediately.

Day 1 Procedure

2 Aspirate Cell-Tak solution if not already done.

3 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.

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

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

6 Prepare LPS: 3 µl of 1 µg/ml LPS in 297 µl stimulation medium

7 Add 20 µl media to wells A-D, H and 20 µl LPS dilution to wells E-G

8 Incubate plate at $37^oC/5\%CO_2$ incubator for 23 hours.

9 Turn on XFp analyzer to warm up overnight

10 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^oC in non-CO_2 incubator.

Day 2 Materials to Prepare

11 Seahorse DMEM Media
Add 50 µl pyruvate, 50 µl L-glutamine, 20 µl glucose to 5 ml Seahorse Base Medium. Sterile filter before use.

Day 2 Procedure

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

13 Aspirate supernatant and replace with 200 µl Seahorse DMEM medium

14 Incubate plate 1 hr at 37^{o}C in non-CO_2 incubator

15 While plate is incubating, complete steps 16-17.

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

17 Set up program on Seahorse XFp and calibrate sensor cartridge (**remove lid**).
Basal: 6 measurements

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

19 Run Assay.

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

Data Analysis

21 Data are analyzed by mean ECAR.