Immobilization of non-adherent cells (Suspension cells을 사용 할 경우) for Seahorse XFe96, Agilent

Suspension cells (ex. Lymphocytes or platelets)을 이용하여 OCR/ECAR측정을 하기 위해서는 adherent monolayer condition을 조성하기 위해 Cell-Tak (Corning, Cat. #354240)과 같은 tissue adhesives가 사용될 수 있습니다. 이 경우, 아래의 실험 protocol대로 진행하시면, suspension cells의 OCR/ECAR측정에 도움이 될 수 있습니다.

Materials

- Agilent Seahorse XF DMEM or RPMI Medium (세포체학실험실에서 50mL 단위 구입 가능)
- Agilent Seahorse XF96 cell culture microplate (세포체학실험실에서 개별 구입 가능)
- Sodium bicarbonate ($NaHCO_3$) (Sigma, Cat. #S5761) -> Working sol. **0.1M (pH 8.0)**
- Sodium hydroxide (NaOH) (Sigma, Cat. # 38215)
- Autoclaved 3DW

Preparation of Cell-Tak solution & application

1) 0.1M Sodium bicarbonate solution (pH 8.0) 2.5mL를 준비한다.
2) Cell-Tak powder 56ug을 준비된 1) 0.1M NaHCO3 (pH 8.0)에 녹인다.
3) Seahorse XFe96 cell culture plate 각 well에 25uL씩 2) Cell-Tak master mix를 넣고 20 min/RT

4) Rinse twice each well with 200uL of 3DW

5) Cell seeding 전까지 4℃ 냉장고에서 보관 가능(less than 1 week)

6) 냉장고에 보관하던 Cell-Tak coated Seahorse XFe96 cell culture plate를 clean bench에서 RT warming up

7) Preparation of 50mL of assay medium (XF base medium + D-glucose, pyruvate and glutamine, if necessary) pH 7.4 with 0.1N NaOH

8) 실험 조건별(condition) 또는 그룹별(pretreatment)로 cell suspension을 준비한다. ex) 1.5 X 10^5 cells/well X 96 well = 144 X 10^5 in conical tube

9) Centrifugation (200 X g/5 min/RT)

10) Remove supernatant

11) Resuspend with 7) "XF base assay medium" 50uL per well. ex) 1.5 X 10^5 cells/well X 96 well X 50uL = 4.8mL

12) Tissue culture reservoir과 multi-pipet을 이용하여 Cell-Tak coated Seahorse XFe96 culture plate에 50uL (1.5 X 10^5 cells/50uL)씩 seeding한다.

(A1, A12, H1, H12 well에는 XF base medium(only medium with no cells)만 적하)

13) Plate centrifugation (200 X g/1min with **zero braking**)

14) Non-CO_2 incubator at 37℃ for 30min

15) 모든 wells에 XF base assay medium 130uL를 넣어주어 total volume을 180uL으로 맞춰준다.

16) Non-CO_2 incubator at 37℃ for 20min

17) Sensor cartridge에 drug treatment를 진행한 후, Seahorse XFe96장비에서 calibration 진행

18) Monitor 안내에 따라 Utility plate를 Cell-Tak coated Seahorse XFe96 cell culture plate로 교체

19) OCR/ECAR reading

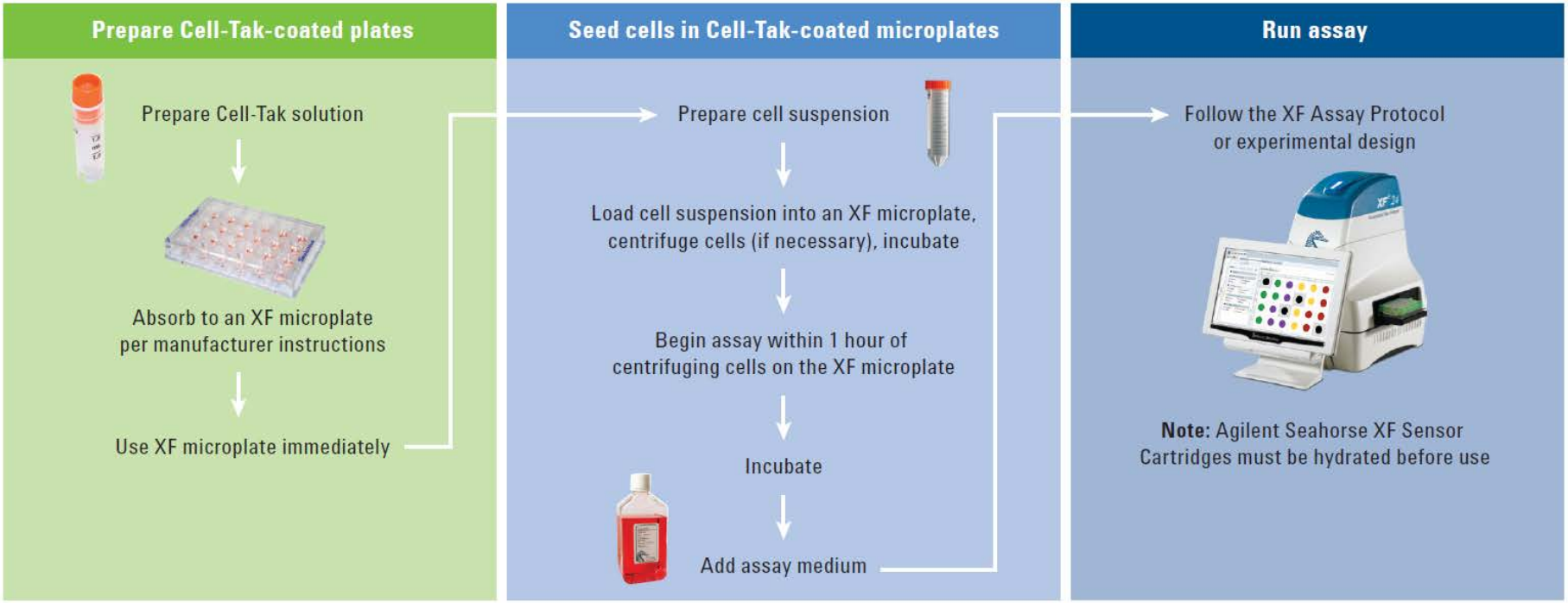

Figure 1. Protocol flow chart.

(Agilent Technical Overview: Immobilization of Non-Adherent Cells with Cell-Tak for Assay on the Agilent Seahorse XFe/XF96 or XFp Analyzer)

Alternative method with Cell-Tak protocol [1]

- ➔ For JLat cells (subclone derived from Jurkat-based cells infected with a pseudotyped human immunodeficiency virus type 1 (HIV-1), Jurkat cells, T-cells
- ➔ A master mix containing Cell-Tak adhesive:

- sodium bicarbonate (75 g/L),
- sodium hydroxide (40 g/L)
- Cell-Tak (2.03 g/L)

at a volume ratio of 291:5:4.

➔ For each Seahorse XFe96 cell culture well, 20 µL of master mix should be added, incubated at room temperature for more than 20 minutes, and completely dried off.

➔ Next, cells should be added to the wells at a concentration of 1.5 x 10^5 cells per well, suspended in 70 µL of DPBS (Do not seed cells on A1, A12, H1, H12 wells for these wells are going to be used for background signal correction)

➔ Incubate the cells at 5% CO2, 37 °C for 60 minutes, and un-adhered cells were carefully washed off with Seahorse XF RPMI media (with D-glucose, pyruvate, glutamine).

➔ Top off each well with 180uL of XF RPMI media

➔ Follow the Seahorse XFe96 OCR/ECAR experiment protocol uploaded in the archive of SNUH Cellomics Core.

How to deal with OCR/ECAR experiment with HEK293T which is easily detached cells

293T (ATCC # CRL-3216)는 parental 293 cells (Human Embryonic Kidney)에 neomycin/G418 (Geniticin) resistant SV40 large T antigen (pRSV-1609) plasmid를 stably transfection함으로써 만들어진 cell line입니다. SV40 large T antigen은 HEK293T cells의 tumor suppressor proteins (p53, p105-Rb)에 결합함으로 HEK293T가 G1 phase에서 S phase로 진행하도록 밀어내며 이를 통해 cell DNA와 함께 incorporation된 viral genome의 replication을 함께 진행하는 것으로 알려져 있습니다 [2-4]. 또한, 세포의 기질 특성을 변화시키는 것으로도 보고되었는데, collagen type IV -> type I으로의 변화와 Na+/K+ pumping

저하와 disruptive effect on actin cytoskeleton 등의 영향으로 cell adhesive가 감소하는 것으로 사료됩니다 [5,6]. 이러한 cell lines (TC treated polystyrene flat bottom plates에 부착은 되나 rinsing과정 또는 drug treatment과정 중에 쉽게 탈락되는 세포주)를 이용하여 Seahorse XFe96에서 OCR/ECAR를 측정하는 경우, Cell-Tak coating, collagen coating 또는 PDL coated plates의 사용 대신 기존에 세포체학실험실에서 진행되었던 아래의 alternative experimental approach를 시도하여 data를 얻는 방법도 있습니다.

방법)

Day-1: Sensor cartridge hydration (Calibrant soaking: 200uL/well, 37℃/non-CO_2 incubation overnight)

D-day:

① Prepare XF assay media (100mL) with D-glucose, pyruvate and glutamate (37℃/non-CO_2 incubator warming-up)

② Conical tube에 HEK293T cells suspension (XF assay media 10mL) -> centrifuge (200 X g/5 min/RT) x 2 times

③ HEK293T cell suspension 1.5 X 10^5 cells/well X 92 well X 180uL = 13.8 X 10^6 cells/16.56mL (XF assay media)

④ Tissue culture reservoir과 multi-pipet을 이용하여 Seahorse XFe96 culture plate에 180uL (1.5 X 10^5 cells/180uL)씩 seeding한다.

(A1, A12, H1, H12 well에는 XF base medium(only medium with no cells)만 180uL 적하)

⑤ Non-CO2 incubator at 37℃ for 60min

⑥ Prepare compound stock solution for A, B, C, D ports ex) oligomycin, FCCP, rotenone and antimycin A

⑦ Seahorse XFe96 sensor cartridge calibration

⑧ OCR/ECAR reading using Seahorse XFe96

위의 방법은 또한, proliferation rate가 다양하며 각기 다른 조건에 노출된 primary cells의 seeding confluency를 맞추어 실험을 진행하기 위한 방법으로도 활용될 수 있습니다. 단, non-adherent cells에서는 활용 될 수 없습니다. 이 경우, 반드시 Cell-Tak coating 또는 PDL coated cell culture plate를 사용하시기 바랍니다.

Reference

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Seahorse XFe96 실험관련 문의: 세포체학실험실(ext. 1714 / e mail: 21117@snuh.org)