Immobilization of Non-Adherent Cells with Cell-Tak for Assay on the Agilent Seahorse XFe/XF24 Analyzer

Technical Overview

Introduction

Cell-Tak cell and tissue adhesive may be used to prepare adherent monolayer cultures of biological samples normally grown in suspension such as lymphocytes and platelets, for assay on the Agilent Seahorse XFe/XF24 Analyzer[1-7]. Cell-Tak is a nonimmunogenic extracellular matrix protein preparation isolated from the marine mussel, *Mytilus edulis*[8].

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The following protocol (Figure 1) is for cells grown in suspension that do not naturally settle to the bottom of the microplate well under gravity, thus requiring centrifugation to settle down. For cells that naturally settle down to the bottom of the well, skip to Seeding Cells in Cell-Tak-Coated Plates, step 11.

Materials

- Agilent Seahorse XF Base Medium (p/n 102353-100 for 2 L; 103193-100 for 100 mL)
- Agilent Seahorse XF24 Cell Culture Microplate (p/n 100777-004)
- Corning Cell-Tak Cell and Tissue Adhesive, 1 mg (Corning, Cat. # 354240)
- Sodium bicarbonate ($NaHCO_3$) (Sigma, Cat. # S5761)
- Sodium hydroxide (Sigma, Cat # 38215)
- Tissue Culture Grade Sterile Water (Invitrogen, Cat. # 15230)
- Water bath set at 37 °C
- Pipettors (single or multichannel)
- Optional, depending on cell type: Benchtop centrifuge with swing-bucket rotor equipped with plate carriers. Example: Eppendorf Centrifuge 5810R.

Preparation of Cell-Tak Coated Plates

Follow the manufacturer's Basic Absorption Coating Protocol, and refer to the Coating Procedure for Multiple Well Plates outlined in the Instructions for Use[8]. Agilent Seahorse Bioscience has identified the following reference points and exceptions helpful to adapt the coating procedure for use with Agilent Seahorse XF24 Cell Culture Microplates:

1. The optimal Cell-Tak solution concentration for the Seahorse XF24 Cell Culture Microplates is 22.4 µg/mL.
2. Prepare 1.5 mL of this solution: Refer to the Manufacturer's protocol to prepare this solution.
3. Apply 50 µL of the solution to each well for 20 minutes at room temperature.
4. Wash each well twice using 200 µL of sterile water
5. Cell-Tak-coated Seahorse XF24 Cell Culture Microplates may be stored for up to one week at 4 °C.
6. Cell-Tak-coated XF24 Cell Culture Microplates must be allowed to warm to room temperature in the hood before cell seeding.

Note: Per manufacturer's Instructions for Use, do not pre-incubate serum-containing medium in the Cell-Tak-coated wells prior to cell seeding, as this may result in a loss of adhesion.

Seeding Cells in Cell-Tak-Coated Plates

Note: Optimal cell density may vary between cell types. Agilent Seahorse recommends optimizing cell density parameters prior to beginning the assay to ensure reproducible results.

The following protocol (Figure 1) describes seeding ONE Agilent Seahorse XF24 Cell Culture Microplate. To balance the centrifuge, create a dummy plate by adding 100 µL of water to each well.

1. Prepare assay medium by supplementing Agilent Seahorse XF Base Medium as required by the experimental conditions. Warm in a 37 °C water bath.

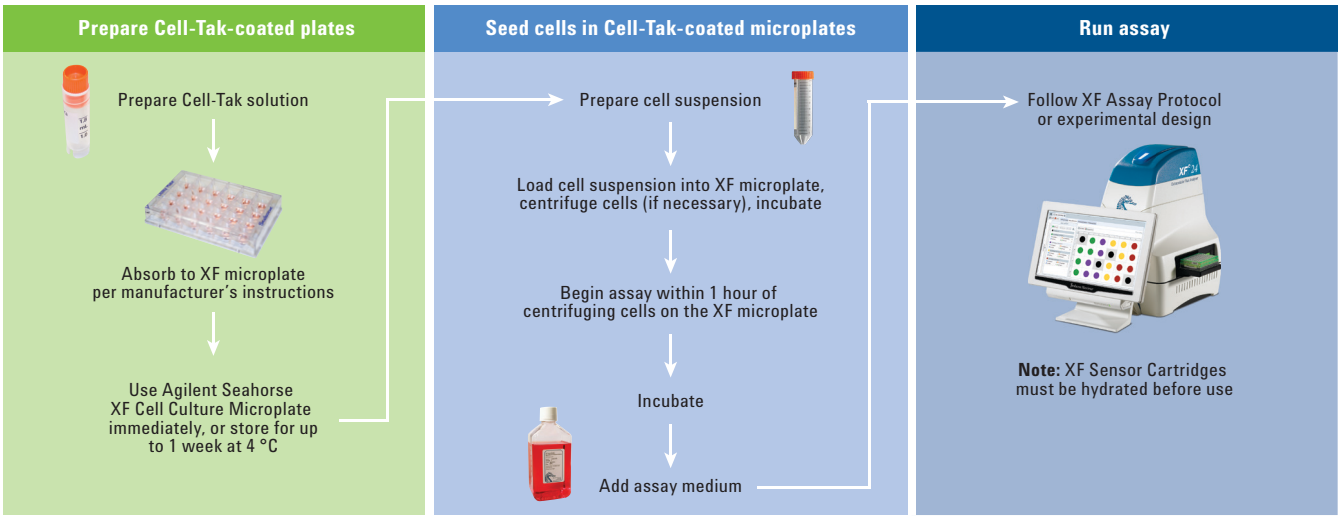

Figure 1. Protocol flow chart.

2. For one Seahorse XF24 Cell Culture Microplate, transfer appropriate volume of cell suspension from the growth vessel to a 50-mL conical tube. To calculate the total number of cells needed, multiply the desired number of cells per well times 25 wells (For example, 150,000 cells per well × 25 wells = 3.75×10^6 cells needed).

3. Centrifuge cells at room temperature at 200 × g for 5 minutes.

4. While cells are being centrifuged, pipette 100 µL assay medium into background/control wells of the room-temperature Cell-Tak-coated XF24 Cell Culture Microplate.

5. Remove supernatant from the centrifuged conical tube.

6. Resuspend cells in an appropriate volume of warmed assay medium that results in the desired number of cells per well in 100 µL of assay medium (For example, if 1.5×10^5 cells per well is desired, resuspend cells in a volume that results in 1.5×10^5 cells/100 µL or 1.5×10^6 cells/mL)

7. Change centrifuge settings to zero braking.

8. Transfer the cell suspension to a sterile tissue culture reservoir or pipette from the conical tube.

9. Pipette 100 µL of the cell suspension along the side of each well, except for background/control wells. Agilent recommends using a multichannel pipette.

10. Centrifuge the cells at 200 × g (zero braking) for 1 minute. Ensure that the centrifuge is properly balanced.

11. Transfer plates to a 37 °C incubator not supplemented with CO_2 for 25–30 minutes to ensure that the cells have completely attached. Visually confirm that most of the cells are stably adhered to the culture surface.

Note: The cells will be morphologically indistinguishable from cells settled on an uncoated Agilent Seahorse XF Microplate. Sensor Cartridge calibration should be started at this time to streamline the assay process.

12. Slowly and gently, add 400 µL warm assay along the side of each well. Be careful to avoid disturbing the cells.

13. Observe the cells under the microscope to check that cells are not detached.

14. Return the cell plates to the incubator for 15–25 minutes.

15. After 15–25 minutes, cell microplates are ready for assay. Total time following centrifugation should be no greater than 1 hour for best results.

16. Place the cell plate in the Agilent Seahorse XFe or XF Analyzer, following calibration.

17. Proceed, following the assay protocol.

Notes

This protocol specifies the full-plate seeding of a single Seahorse XF24 Cell Culture Microplate. If more than one plate is desired, increase the volumes and total cell numbers required proportionately. Agilent Seahorse recommends seeding two plates when beginning work with a cell line for additional practice with step 12 (addition of medium without disrupting cells).

This cell seeding protocol was developed by Agilent Seahorse Bioscience scientists, and is applicable exclusively to cells cultured in Seahorse XF24 Cell Culture Microplates coated with Cell-Tak and intended for analysis using the Seahorse XFe or XF24 Analyzer.

References

1. Wang, R.; *et al. Immunity* **2011**, *35(6)*, 871-82 (T-lymphocytes).
2. Capasso, M.; *et al. Nat. Immunol.* **2010**, *11(3)*, 265-72 (B-lymphocytes).
3. Avila, C.; *et al.* Exp. *Clin. Endocrinol. Diabetes* **2011**, DOI: 10.1055/s-0031-1285833 2011 (platelets).
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5. Rogers, G.; *et al.* PLoS One **2011**, *6(7)*, e21746 (isolated mitochondria).
6. Bulua, A. C.; *et al. J. Exp. Med*. **2011**, *208(3*), 519-33 (PBMC).
7. Saha, A.; *et al. Gut* **2010**, *59(7)*, 874-881(AGS adenocarcinoma cells).
8. Corning Cell-Tak Cell and Tissue Adhesive Instructions for Use.

Further Reading

Agilent Application Note (available online):

Bioenergetic analysis of suspension cells: hematopoietic stem cells and lymphocytes. A real-time assay that quantifies the ATP and biosynthetic demands of immune cell proliferation, differentiation, and effector function.

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