Washing Cells in Seahorse XFp Cell Culture Miniplates

Before performing an XF assay, growth medium must be replaced with a suitable assay medium (generally this means medium without bicarbonate buffer or serum and with low phenol red content). This procedure describes replacing the growth medium with assay medium for adherent cells grown in Seahorse XFp Cell Culture Miniplates prior to being assayed using a Seahorse XFp Analyzer.

Materials Required:

- Prepared assay medium. See the Basic Procedure for Assay Media Preparation for details on choosing and preparing the assay medium.
- Multi-channel pipette, 200 µL capacity, with matching tips
- Tissue culture microscope
- Non-CO_2 incubator

Procedure:

1. Warm the assay medium to 37°C.
2. Retrieve the Seahorse XFp Cell Culture Miniplate(s) from the tissue culture incubator. You may wish to keep the miniplate(s) in the Seahorse XFp Carrier Tray for ease of handling.
3. Look at the cells under the microscope to:
    a. Confirm cell health, morphology, and purity (no contamination).
    b. Ensure that the cells are adhered and appear as a consistent monolayer.
    c. Make sure that the background wells (A and H) contain no cells.
4. Wash the cells with assay medium:
    a. Remove all but 20 µL of the culture medium from each well. The small amount of medium is left to keep the cells from drying out.
    b. Gently add approximately 200 µL of assay medium, then remove the same amount.
    c. Repeat step 4b, removing all but 20 µL (as in step 4a).
    d. Add assay medium to a total volume of 180 µL – or the volume recommended by the particular assay protocol you are using.
5. Observe the assay wells under the microscope to ensure that cells were not washed away.
6. Place the plate in a 37°C incubator **without CO_2** for one hour prior to the assay.

Incubating the cell plates without CO_2 allows outgassing from the plate and is required for accurate ECAR measurements.