Washing Adherent Cells in Agilent Seahorse XFp Cell Culture Miniplates

Basic Procedure

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/no phenol red content). This procedure describes replacing the growth medium with assay medium for adherent cells grown in Agilent Seahorse XFp Cell Culture Miniplates prior to being assayed using an Agilent Seahorse XF HS Mini or XFp Analyzer.

Materials Required

1. Prepared assay medium. For choosing and preparing the appropriate assay medium, please see http://www.agilent.com/cs/library/selectionguide/public/5991-7878EN.pdf and http://www.agilent.com/cs/library/usermanuals/public/Media%20Prep%20XFp.pdf.
2. Multi-channel pipette, 200 µL capacity, with matching tips
3. Tissue culture microscope
4. Non-CO_2 incubator

Procedure

1. Warm the assay medium to 37˚C.
2. Retrieve the XFp Cell Culture Miniplate(s) from the tissue culture incubator.

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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. Rinse cells two times with 200 µL of assay medium, leaving behind 20 µL each time.
   c. Add 160 µL of assay medium to each well for a final volume of 180 µL/well.
5. Visualize the assay wells under the microscope to ensure that cells were not disturbed or washed away.
6. Place the plate in a 37˚C incubator without CO_2 for 45–60 minutes prior to the assay.

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

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www.agilent.com/en-us/promotions/seahorse-xf-technology

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