Washing Adherent Cells in Agilent Seahorse XF96 Cell Culture Microplates

Basic Procedure

Before performing an Agilent Seahorse 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 XF96 Cell Culture Microplates prior to being assayed using an Agilent Seahorse XF^{e}96/XF96 Analyzer.

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/XFe96_DAY_OF_MEDIA_PREP.pdf or http://www.agilent.com/cs/library/usermanuals/public/XF96_DAY_OF_MEDIA_PREP.pdf

1. Warm the assay medium to 37˚C.
2. Retrieve the XF Cell Culture Microplate plate from the CO_2 incubator.
3. Look at the cells under the microscope to:
   a. Confirm cell health, morphology, seeding uniformity and purity (no contamination).
   b. Ensure cells are adhered, showing a consistent monolayer.
   c. Make sure no cells were plated in the background correction wells.

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4. Wash cells with the appropriate assay medium
   a. Using an Agilent Seahorse XF Prep Station
      i. Attach bottle of assay medium to XF Prep Station. Open the XF Prep Station software. On the "Media Change" tab, select "Do Prime", set final volume to 180 µL of assay medium, and unselect "Do Rinse".
      ii. Place the cell plate vertically onto the tray and remove the lid.
      iii. Press "Start".
   b. Without using an Agilent Seahorse XF Prep Station
      i. Remove all but 20 µL of the culture medium from each well.
      ii. Rinse cells two times with 200 µL of assay medium, leaving 20 µl behind after each wash.
      iii. Add 160 µL of assay medium to each well for a final volume of 180 µL/well.
5. Look at cells 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.

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

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