Washing Adherent Cells in Agilent Seahorse XF24 Cell Culture Microplates

Basic Procedure

Before performing an Agilent Seahorse XF Assay, growth medium must be replaced with a suitable assay medium (generally, medium without bicarbonate buffer or serum and low/no phenol red content). This procedure describes replacing the growth medium with assay medium for adherent cells grown in XF24 Cell Culture Microplates prior to being assayed using an Agilent Seahorse XF^{e}24/XF24 Analyzer.

1. Warm the assay medium to 37˚C.
   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/XFe24_DAY_OF_MEDIA_PREP.pdf or http://www.agilent.com/cs/library/usermanuals/public/XF24_DAY_OF_MEDIA_PREP.pdf.
2. Retrieve the cell culture microplate from the CO_2 incubator.
3. Look at cells under the microscope to:
   a. Confirm cell health, morphology, seeding uniformity and purity (no contamination).
   b. Ensure cells are adhered, and no gaps are present.
   c. Make sure no cells were plated in the background correction wells
4. Wash cells with assay medium
   a. Remove all but 50 µL of the culture medium from each well.
   b. Rinse cells two times with 1 mL of assay medium, leaving behind 50 µl after each wash.
   c. Add 450 µL of assay medium to each well for a final volume of 500 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

Published in the USA, June 30, 2017

Agilent Technologies