Washing Cells in XF96 Cell Culture Microplates

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 XF^{e}96 or XF96 Analyzer.

1. Warm the pre-made assay medium to 37^{o}C. See basic procedure for Assay Media Preparation.
2. Retrieve your XF cell cartridge 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, and no gaps are present.
    c. Make sure no cells were plated in the background correction wells.
4. Wash cells with assay medium (based on the type of XF assay you are running, use XF Cell Mito Stress Test Assay Medium or XF Glycolysis Stress Test Assay Medium)
    a. Using a XF Prep Station
        i. Attach bottle of assay medium to XF Prep Station. Open the Seahorse XF Prep Station software. On the “Media Change” tab, select “Do Prime”, set final volume to 175 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 a 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.
        iii. Add 155 µL of assay medium to each well for a final volume of 175 µL/well.
5. Look at cells 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.