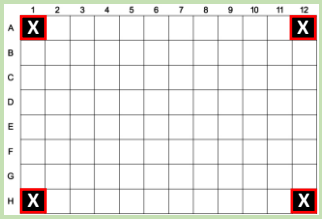
Seahorse XFe96 easy-to-follow guide

D-2

Cell seeding
(4,000 – 8,000 cells/well)



X A1, A12, H1, H12 calibration well only (No cell seeding) Instead, top off with media

D-1

Sensor cartridge hydration
(Calibrant soaking: 200µL/well, **37°C/non-CO_2** overnight)

Seahorse XFe96
Turn ON (overnight)

Wave software ON
Input experimental design

Step exchangeable

D-day

Prepare **XF assay media**
(50mL/plate, **37°C/non-CO_2**)

Prepare compound stock solution (A, B, C or A, B)

Dispense compound stock solution into drug ports (A, B, C, D) -> Keep it under **37°C/non-CO_2** (30 min)

Wave software ON
Input experimental design

Start Run: sensor cartridge calibration (30 min)

Cell culture plate -> XF assay media change

1. **Rinse 2 times** with 100µL/well of XF assay media or 1X PBS
2. **Top off each well** with **180 µL of XF assay media** **37°C/non-CO_2** (40 min)

Phase contrast imaging with Cytation1 (20 min)

Plate switching (calibration plate -> cell culture plate)

Click "I'm ready" (1 hr 40 min)

Normalization (20 min)

Data export (Excel)