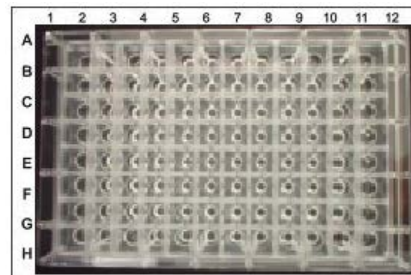


# Seeding Cells in XF<sup>e</sup>96 Cell Culture Microplates

XF assays are performed in a Seahorse 96-well XF Cell Culture Microplate in conjunction with an XF<sup>e</sup>96 sensor cartridge. Each microplate is formatted in a typical 96-well design, as shown. The seeding surface of each well is 0.106 cm<sup>2</sup>, smaller than in a typical 96-well plate, but larger than in a typical 384 well plate. This procedure describes recommendations for seeding adherent cell types for use with the XF<sup>e</sup>96 Analyzer.



1. Harvest and re-suspend the cells to desired final concentration to seed in 80  $\mu$ L of growth medium. Optimal cell seeding numbers vary widely, though are typically between 5,000 – 40,000 cells per well and must be determined empirically.
2. Seed 80  $\mu$ L of cell suspension per well (as shown in figure below); do not seed cells in background correction wells (A1, A12, H1, H12). Be sure to put medium only (no cells) in the background correction wells.
3. Allow plate to rest at room temperature in the tissue culture hood for one hour. This will promote even cell distribution and reduce edge effects. Monitor adherence using a microscope.
4. Allow the cells to grow overnight in a cell culture incubator. Monitor growth and health of cells using a microscope.

**Hint:** Hold the pipette tip at an angle about halfway down the side of the wells for best technique and most homogeneous cell layer.

