

Cell Proliferation Assays:

How much inoculum should be used in ECIS wells?

As in any proliferation assay, the amount of cells used in the inoculum depends on the cell type and the run time of the assay. Below are suggested guidelines on how to achieve a confluent layer of cells, assuming the generation time of the cells being used is about 24 hours (typical for mammalian cells under good growth conditions).

1. For a proliferation assay that runs 2 days to reach confluence, the assay should be inoculated with approximately 4-fold fewer cells than would be found in the confluent layer.
2. For a proliferation assay that runs 3 to 4 days to reach confluence, the assay should be inoculated with approximately 10-fold fewer cells than would be found in the confluent layer.

These two situations are typical examples; but other starting conditions, both higher and lower numbers of cells, can be used. Below are suggested inoculations for assays of different duration using 8 well and 96 well plates. The numbers assume approximately 80% survival of cells upon trypsinization.

For 8 Well Plates:

The area of the 8 well bottom is approximately 0.8 cm^2 . Assuming a typical confluent layer of cells is 1×10^5 cells per cm^2 , 1×10^5 cells are added to the well to achieve confluence without cell proliferation (400 μL of a 2.5×10^5 cells/mL suspension).

2 Days to Confluence

Add 400 microliters of a 6.0×10^4 cells/mL suspension to the empty well. (This is equivalent to 2.4×10^4 cells in the well.)

3 to 4 Days to Confluence

Add 400 microliters of a 2.5×10^4 cells/mL suspension to the empty well. (This is equivalent to 1.0×10^4 cells in the well.)

For 96 Well Plates:

The area of the 96 well bottom is about 0.3 cm^2 . Again, assuming a typical confluent cell layer is 1×10^5 cells per cm^2 , 0.38×10^5 cells are added to the well to achieve confluence without cell proliferation (300 μL of a 1.3×10^5 cells/mL suspension).

2 Days to Confluence

Add 300 microliters of a 3.0×10^4 cells/mL suspension to the empty well. (This is equivalent to 0.9×10^4 cells in the well.)

3 to 4 Days to Confluence

Add 300 microliters of a 1.2×10^4 cells/mL suspension to the empty well. (This is equivalent to 0.36×10^4 cells in the well.)

