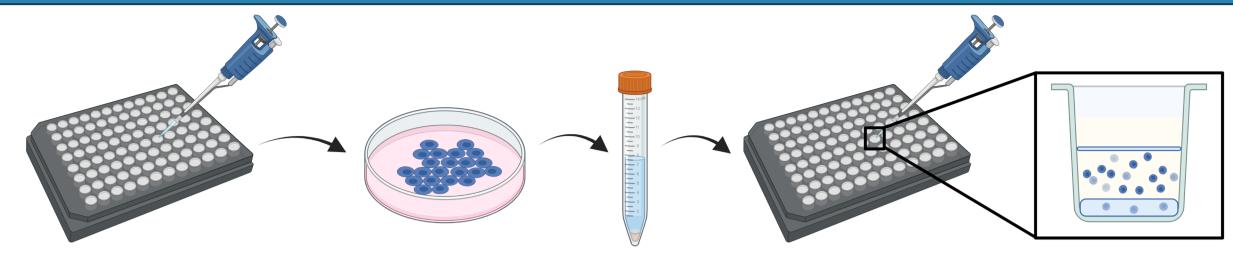
## Day 0

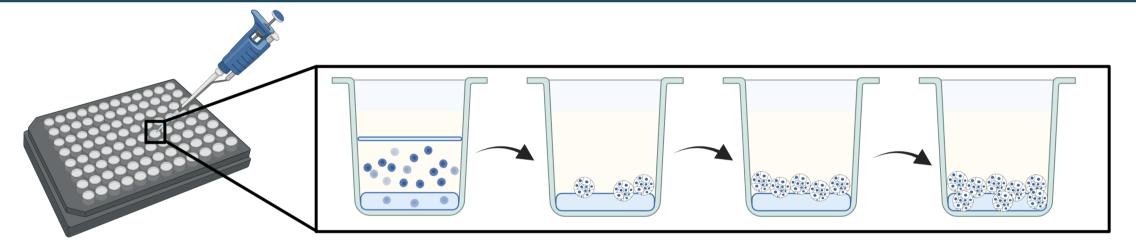


ECM basement material is diluted to the appropriate concentration and plated into a 96-well plate

Monolayer cells are passaged and diluted in phenol red-free medium

Cells are plated at the appropriate concentration into the ECM basement material-coated wells

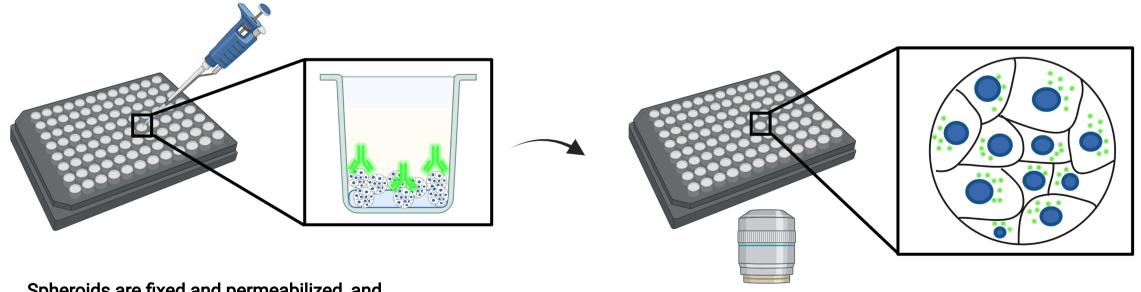
## Day 1 - Day 7 (Cell Line Dependent)



Medium is replaced the following day and every second day thereafter

Spheroid growth is observed each day

## Fixing, Processing and Imaging



Spheroids are fixed and permeabilized, and immunostained with fluorescently-labeled antibodies against specific organelle markers

Spheroids are imaged using high-resolution objectives