In vitro biofilm

Day 1:
Start YPD culture
Autoclave small silicone disks
Autoclave or bake (235C overnight) 100 ml beaker (red)
If doing Glucatell, be sure to use glucan free tips and tubes (orange) and use RPMI/MOPS and not RPMI/HEPES

Day 2:
1. Place 20 µl serum on silicon disk in sterile petri dish and spread.
2. Incubate 37˚ C for 30 min.
3. Adjust cells to 10^6 in RPMI/MOPS using hemacytometer
   cells= # cells/5 small boxes 5 x 10^4 x dilution factor
4. Add 100 µl uridine for 1 ml RPMI if auxotrophic.
5. Inoculate 200 µl culture to top and 200 µl to bottom of silicon disk and spread.
6. Incubate 37˚ C for 1 h.
7. Rinse in Petri dish of sterile PBS
8. Place disk in beaker with 2.5 ml RPMI (add 0.25 ml uridine if auxotrophic).
9. Cover with sterile foil and parafilm
10. Incubate at 37˚ C shaker for at 50 RPM for time period (24 h or 48 h).

Harvest in vitro biofilm

1. Rinse silicon disk in ddH2O and place in 5 ml ddH2O in 50 ml tube.
2. Vortex and scrape silicon surface to remove biofilm cells with spatula.
3. Sonicate 10 minutes.
4. Remove disks from tube.
5. Centrifuge cells 4500g X 20 minutes at 4C. Supernatant contains matrix. Cell pellet is biofilm cells.
6. Remove top 4 ml of supernatant and place in 15 ml tube.
7. Centrifuge supernantant 4500g x 20min for additional 2 times, leaving bottom 0.5 ml on transfer.
8. Final supernatant fraction is matrix. Plate (1, 1:10, 1:100) counts to determine if matrix is cell free. Store -20C
9. Transfer biofilm cell pellet to microfuge tube (pre-weighed) and spin 7000g X 10 minutes.
10. Remove supernatant and resuspend pellet in ddH2O.
11. Obtain viable count dilutions (1:10^3, 1:10^4, 1:10^5, and 1:10^6) is necessary.
12. Spin 7000g x 10 min.
13. Store cell pellet -80C.