

In vitro biofilm (48 hour)

1. Place 50 μ l serum on 5 cm silicon disk and spread.
2. Incubate 37° C for 30 min.
3. Inoculate 50 μ l overnight culture to underside of silicon disk.
4. Inoculate 100 μ l overnight culture to top of silicon disk and spread.
5. Add 1 ml RPMI (add 100 μ l uridine if auxotrophic).
6. Incubate 37° C for 45 min.
7. Place disk in beaker with 10 ml RPMI (add 1ml uridine if auxotrophic).
8. Incubate in 37° C water bath with shaker for 24 hours.
9. Rinse disk sterile ddH₂O and place in 10 ml RPMI (add 1ml uridine if auxotrophic).
10. Incubate in 37° C water bath with shaker for 24 hours.

Harvest in vitro biofilm

1. Rinse silicon disk in ddH₂O X 2 and place in 30 ml ddH₂O in 50 ml tube.
2. Scrape silicon surface to remove biofilm cells with spatula.
3. Sonicate 10 minutes.
4. Remove disks from tube.
5. Centrifuge cells 2600g X10 minutes.
6. Wash with 10 ml ddH₂O and centrifuge 2600g X 10 minutes.
7. Remove supernatant and resuspend in remaining H₂O.
8. Transfer to microfuge tube.
9. Microcentrifuge 4000 RPM X 5 minutes and remove supernatant.
10. Store cells -80° C.

