12-Well Plate In Vitro Biofilms (BWP17 strains)

1. Grow up an overnight 3 ml planktonic culture in YPD.
2. Coat each well of plate with 10 ul serum, using a spreader.
3. Incubate at 30˚C for 30 minutes, without shaking or parafilm.
4. Count cells from overnight culture and adjust to 1 X 10^6 in RPMI/MOPS (Karen's).
5. Add 500 ul of adjusted cells to each well, making sure to coat the bottom, and incubate at 30˚C for 45 minutes without shaking.
6. Remove media from wells.
7. Add 500 ul fresh RPMI to each well, wrap plate in parafilm then foil, and incubate at 37˚C with shaking at 50 RPM.
8. For harvesting, remove media from wells and wash with 1 ml PB1.
Add 100 ul of 1XPBS or H2O to each well and scrape with a scraper. Repeat with additional 100 ul. Transfer to an appropriate tube and store as needed.

12-Well Plate In Vitro Biofilms (K1)

1. Grow up an overnight planktonic culture in RPMI/MOPS from plate. Do not start directly from frozen stock.
2. Coat each well of plate with 10 ul serum and spread.
3. Incubate at 30˚C for 30 minutes, without shaking or parafilm.
4. Dilute overnight culture 1:1 with fresh RPMI/MOPS (Karen's) and vortex.
5. Add 500 ul of adjusted cells to each well, making sure to coat the bottom, and incubate at 30˚C for 45 minutes without shaking.
6. Remove media from wells.
7. Add 500 ul fresh RPMI to each well, wrap plate in parafilm then foil, and incubate at 37˚C with shaking at 50 RPM.
8. For harvesting, remove media from wells and wash with 1 ml PBS or H2O.
Add 100 ul of 1XPBS or H2O to each well and scrape with a scraper. Repeat with additional 100 ul. Transfer to an appropriate tube and store as needed.

For 6 well biofilms: Increase volumes by 2

Matrix Isolation
1. Growth K1 biofilms as describe above. Use H2O, not PBS for washing and harvesting. Place in 15 ml conical tube.
2. Vortex and Sonicate 10 minutes.
3. Centrifuge 4500g for 20 minutes at 4C. Transfer all liquid except bottom 1.5 ml liquid and cell pellet using 5 ml pipette.
4. Repeat centrifugation on transferred liquid at 4500g for 20 minutes at 4C. Again, transfer all liquid except bottom 1.5 ml liquid using 5 ml pipette.
5. Repeat step 4. (Total centrifugations is 3).
6. Place in 50 ml conical tube and store at -80C for lyophilization. Tubes may be filled up to half full.