Culture media:

|  |  |  |  |
| --- | --- | --- | --- |
| Reagent | Stock concentration | Concentration in culture | For 50mL mastermix |
| Cyclohexamide | 10mg/mL | 20ug/mL | 100uL |
| Hutners trace | 1000x | 1x | 50uL |
| 0.5X PBS | 0.5x | 0.5x | 44.85mL |
| NaNO3 | 100mM | 5mM | 2.5mL |
| (NH4)2SO4 | 100mM | 5mM | 2.5mL |

100mM Sodium nitrate (85gmol) – 0.425g in 50mL 0.5x PBS

100mM Ammonium sulfate (132gmol) – 0.66g in 50mL 0.5x PBS

250uL total culture volume per well – headspace = 1mL

1% substrate, e.g. 2.5mg biofilm per 250uL culture, for 20 wells, make a mastermix of 50mg biofilm + 5mL culture media