|  |  |  |  |
| --- | --- | --- | --- |
| 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 | 49.85mL |

3x replicates of 1%, 5%, 10% inoculum (10uL, 50uL, 100uL) for total volume 1mL culture

In 96 well plate add,

990, 990, 990, 950, 950, 950, 900, 900, 900 mastermix, final 3 wells 1mL 1mL 1mL (glucose)

Second row 1mL, 1mL, 1mL negative control

Layout A1, A2 and A3 = 1% 25mg

A4, A5, and A6 = 5% 25mg

A7, A8, and A9 = 10% 25mg

A10, A11 and A13 = 10% Glu 25mg ctrl

B10, B11, B13 = 0% Glu 25mg ctrl

B5, B6, B7 = 10% ctrl 0mg

1mL is too much, reduce to 800uL total culture volume

25mg substrate