**Minimal Inhibitory Concentration (MIC)**

**Purpose:** To determine the lowest concentration of cobalt that *Delftia acidovorans* cannot survive in

**Methods:**

1. Obtain a 96-well plate
2. Label as follows:

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | TSB | TSB+C | 30mM | 15mM | 7.5mM | 3.75mM | 1.88mM | 0.94mM | 0.47mM | 0.23mM | 0.12mM | 0.06mM |
| A |  |  |  |  |  |  |  |  |  |  |  |  |
| B |  |  |  |  |  |  |  |  |  |  |  |  |
| C |  |  |  |  |  |  |  |  |  |  |  |  |
| D |  |  |  |  |  |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |  |  |  |  |  |
| F |  |  |  |  |  |  |  |  |  |  |  |  |
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

1. Add 100μL of overnight culture to 9.9mL TSB in a reservoir
2. Using a multichannel pipette, add 100μL TSB+C to columns 2-12
3. Prepare a 60mM Cobalt solution (for 30mM final solution in column 3)
	1. In an Eppendorf microcentrifuge tube:

\*60μL of 1M cobalt stock

\*10μL of overnight culture

\*930μL of TSB

1. Add 100μL of 60mM Cobalt solution to all wells in column 3
2. Using a multichannel pipette, pipette column 3 up and down 3 times to mix, and then add 100μL of column 3 solution (30mM) to column 4 to create a 15mM solution
3. Repeat step 7 all the way down the plate
4. Take up 100μL of column 12 solution and discard with tips to ensure consistent volume in all wells
5. Incubate plate at 30 oC overnight

\*Procedure was repeated with each isolate of *Delftia acidovorans*

\*Note – Procedure was performed with Cobalt, but can also be performed with other heavy metals