## Culturing of Dethiobacter alkaliphilus

Strain AHT1T was grown anaerobically at 30 °C in Na carbonate buffered mineral medium (22 g/L Na2CO3, 8 g/L NaHCO3, 6 g/L NaCl, 1 g/L K2HPO4) with a pH of 10 and 0.6 M total Na+. Additionally, 4 mM NH4Cl, 1 mM MgCl2 x 6H2O and 1 mlL−1 trace element solution were added [18]. After sterilization, hypotaurine(HT) serving as a carbon source (1 mM) and KNO3 (10 mM) the electron-acceptor, were also added to the medium. The medium will be inoculated with Dethiobacter alkaliphilus before transferring it into 24 well-plates for cell culturing. The plate will be incubated in an anaerobic environment where candles are placed in the incubation tank to deplete all oxygen supply.

Preparation of Na carbonated buffer mineral medium

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| --- | --- |
| Compound | Weight (g/L) |
| Na2CO3 | 22 |
| NaHCO3 | 8 |
| NaCl | 6 |
| K2HPO4 | 1 |

Additional trace element solution

|  |  |
| --- | --- |
| Compound | Converted measurement |
| 4 mM NH4Cl | 0.004m/L, if dissolving pure NH4Cl, **0.213964g** is needed for 1l of solution.  Molecular weight of NH4Cl x 0.004 = 0.213964g  53.491 g/mol x 0.004 m/L = 0.213964g |
| 1 mM MgCl2 x 6H2O | 0.001m/L, if dissolving premade MgCl2 x 6H2O, **0.2033g** is needed for 1l of solution.  Molecular weight of MgCl2 x 6H2O x 0.001= 0.2033g  203.30 g/mol x 0.001 m/L = 0.2033g |
| 1 mlL−1 trace element solution | 1ml is needed for 1l of solution |

\*Note about MgCl2, it is very difficult to accurately measure magnesium chloride as it tends to absorb moisture from the air, hence it is recommended to use MgCl2 x 6H2O

Carbon source

|  |  |
| --- | --- |
| Compound | Converted measurement |
| 1mM hypotaurine | 0.001m/L, if dissolving hypotaurine, **0.109g** is needed for 1l of solution.  Molecular weight of hypotaurine x 0.001= 0.109g  109.147 g/mol x 0.001 m/L = 0.109g |
| 10 mM KNO3  \*Note KNO3 is moderately soluble in water | 0.010m/L, if dissolving KNO3, **1.011g** is needed for 1l of solution.  Molecular weight of KNO3 x 0.01= 1.011g  101.1032 g/mol x 0.010 m/L = 1.011g |

**Possible protocol**

* Weigh out 22g of Na2CO3, 8g of NaHCO3, 6g of NaCl and 1g of K2HPO4 to one litre of milli-q water.
* Add 4 mM of NH4Cl, 1 mM of MgCl2 x 6H2O and 1 mlL−1 trace element solution according to the table above.
* Filter the buffer solution through a 0.22 μm filter into a sterile flask/bottle (preferably autoclaved prior) or autoclave for 15 to 20 minutes. \*Seems like one cycle would be more logical and safer
* Let the autoclaved solution cool down.
* Add 1 mM hypotaurine and 10 mM potassium nitrate according to the calculation in the table above.
* Filter the final solution through a 0.22 μm filter into a sterile bottle for storage (optional but would be good)
* Pipette out 50ml of the buffer into a falcon tube and inoculate Dethiobacter alkaliphilus by suspending it in the buffer.
* Transfer 500μL of the culture into each of the 24 well plate
* Place the 24-well plate into a 28°C incubator that has candles placed in it 30 minutes prior to the incubation.