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🌐 Motility Medium(Buffer)

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ABSTRACT

MM for bacterial motility experiments

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Protocol status: Working
We use this protocol and it's working

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Prepare Stock Solutions

1 Potassium Phosphate(KPO₄) Stock Buffer [1M] (100x):

1-) Prepare 1M Potassium Phosphate Monobasic

- 500 mL ddH₂O (milliQ)
- 68.045 gr of Monobasic potassium phosphate (KH₂PO₄) [1M]
- 87.09 gr of Dibasic potassium phosphate (K₂HPO₄) [1M]

2-) Adjust the pH of the solution to 7.0

3-) Filter sterilize.

4-) Store at room temperature(+4C).

L-methionine Stock Buffer [10mM] (10000x):

1-) Mix the ingredients

- 500 mL ddH₂O (milliQ)
- 746 mg L-methionine

2-) Stir the mix until L-methionine dissolve.

3-) Check the pH and adjust to 7.0

4-) Filter sterilize.

5-) Store in the cold room(+4C).

Note: You have to dilute this stock solution down to 100μM before adding into motility medium mix.

Lactic Acid Stock Buffer [1M](100x):

1-) Mix the ingredients

- 46.27 mL ddH₂O (milliQ)
- 3.73 mL Lactic Acid (d = 1.209 g/mL)

2-) Briefly stir

3-) Check the pH and adjust to 7.0

4-) Filter sterilize.

5-) Store in the cold room(+4C).

EDTA Stock Buffer[10mM](100x):

1-) Mix the ingredients

- 49 mL ddH₂O (milliQ)

- 1 mL EDTA [0.5M]

2-) Briefly stir.

3-) Filter sterilize.

4-) Store in the cold room(+4C).

Note

Since the masses of monobasic and dibasic potassium phosphate are large, they take up considerable amount of volume inside the buffer.

To get the correct molar concentration of both compounds inside the buffer, first add chemicals inside an empty beaker and only then add the ddWater and complete the total volume of the buffer to 500mL.

Prepare Motility Medium

2 1-) Mix the following ingredients:

- 480 mL ddH₂O(milliQ)

- 5 mL Potassium Phosphate(KPO₄) [1M] (100x)

- 5 mL L-Methionine [**100 µM**] (100x)

- 5 mL Lactic Acid [1M] (100x)

- 5 mL EDTA [10mM] (100x)

- 67 µL NaCl [1M]

2-) Briefly stir.

3-) Adjust the pH to 7.0

4-) Filter sterilize.

5-) Store in the cold room(+4C).

Note

The stock solution of L-Methionine is **1mM(1000μM)**. You must dilute this down to **100μM** before adding into the motility buffer.