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Motility Medium(Buffer)

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1 Works for me dx.doi.org/10.17504/protocols.io.mx8c7rw

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ABSTRACT

MM for bacterial motility experiments

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Prepare Stock Solutions

1 Potassium Phosphate(KPO_4) Stock Buffer **[1M] (100x)**:

1-) Prepare 1M Potassium Phosphate Monobasic

- 500 mL ddH_2O (milliQ)
- 68.045 gr of Monobasic potassium phosphate (KH_2PO_4) [1M]
- 87.09 gr of Dibasic potassium phosphate (K_2HPO_4) [1M]

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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 ddH_2O (milliQ)
- 746 mg L-methionine

2-) Stir the mix until L-methione 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 ddH_2O (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 ddH_2O (milliQ)
- 1 mL EDTA [0.5M]

2-) Briefly stir.

3-) Filter sterilize.

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

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.

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Prepare Motility Medium

2 1-) Mix the following ingredients:

- 480 mL ddH_2O(milliQ)
- 5 mL Potassium Phosphate(KPO_4) [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).

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

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