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iGEM TU/e 2015
Biomedical Engineering

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Alginate beads

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Alginate beads

1 Preparation NaCl solution (0.9%)

Estimated bench time: 15 minutes
Estimated total time: 15 hours
Purpose: This solution is used for dissolving the bacteria.

1.1 Materials

- 0.22 µm filter
- Balance
- Falcon tube
- MiliQ
- NaCl (Sodium chloride)
- Pipette and tips
- Syringe
- Vortex

1.2 Setup & Protocol

- Dissolve 0.18 g NaCl in 20 ml MiliQ.
- Mix/vortex so that all the NaCl goes into solution.
- Filter into a Falcon tube using a syringe and a 0.22 µm filter for sterilization.

2 Preparation alginate solution (2.5%)

Estimated bench time: 10 minutes
Estimated total time: 3 hours
Purpose: This solution forms, together with the bacteria, the basis of the alginate beads.

2.1 Materials

- Alginate
- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- NaCl solution (0.9%)
- Pipette and tips
- Mixing magnet

2.2 Setup & Protocol

- Dissolve 0.25 g alginate slowly in 10 ml 0.9% NaCl solution. During the entire dissolving process a mixing magnet must be used to keep mixing the solution.
- Autoclave the alginate solution at 121 °C for 20 minutes (sterilization).

3 Preparation of $CaCl_2$ solution (100 mM)

Estimated bench time: 15 minutes
Estimated total time: 15 hours
Purpose: This solution is used for creating the beads after the alginate droplets with bacteria are made.

3.1 Materials

- 0.22 µm filter
- Balance
- Beaker (glassware)
- $CaCl_2$
- Falcon tube
- MiliQ

3.2 Setup & Protocol

- Dissolve 1.1098 g $CaCl_2$ in 100 ml MiliQ.
- Mix so that all the $CaCl_2$ goes into solution.
- Filter into a Falcon tube using a syringe and a 0.22 µm filter for sterilization.

4 Preparation bacteria

Estimated bench time: 20 minutes
Estimated total time: 30 minutes
Purpose: The bacteria are transferred from the LB medium to a NaCl solution..

4.1 Materials

- Cell Density Meter (OD600)
- Cuvette
- LB medium
- LB medium with bacterial cells
- NaCl solution (0.9%)
- Pipette and tips
- Tabletop Centrifuge

4.2 Setup & Protocol

- Pipette 1 ml of the LB medium with bacterial cells in the cuvette and measure the OD600. The OD measurement requires a blank measurement with 1 ml LB medium.
- If the OD600 is at least 1.5, continue, else wait and preform new measurement.
- Spin down the bacteria by centrifuging for 10 minutes at 4,000 rpm. Weight balance well.
- Discard the supernatant and resuspend the pellet with 1 ml of 0.9% NaCl solution.

5 Alginate beads

Estimated bench time: 15 minutes
Estimated total time: 15 minutes
Purpose: Creating alginate beads which will contain bacteria.

5.1 Materials

- Alginate solution (2.5%)
- Bacteria in NaCl solution (0.9%)
- $CaCl_2$ solution (100 mM)
- Falcon tube
- Pipette and tips
- Syringe (sterile)

5.2 Setup & Protocol

- Mix 1 ml bacteria in 0.9% NaCl solution with 4 ml alginate solution, this means going from 2.5% to a 2% alginate solution.
- Put the suspension in a sterile syringe and use it to put droplets in 100 ml of the 100 mM $CaCl_2$ solution.
- Put the alginate beats in a Falcon tube for storage.

6 (Optional) View beads

Estimated bench time: 10 minutes
Estimated total time: 10 minutes
Purpose: Confirming that the alginate beats contain the bacteria by showing the fluorescence proteins.

6.1 Materials

- Alginate beats
- Blue lamp
- Blue lamp protecting glasses
- Falcon Tube

6.2 Setup & Protocol

- Place the Falcon tube with the alginate beads on a blue lamp. Use protecting glasses.
- Make a picture of the alginate beats showing fluorescence.