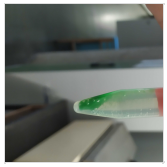

Version 2

Oct 04, 2021

Cyanobacteria Yolk-Shell Preparation V.2

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ABSTRACT

Cyanobacteria as well as other photosynthetic microorganisms can be encapsulated within biohybrid nanomaterials. Cyanobacterial Yolk-shell are biohybrid nanostructures with a size of 1 to 6 µm, where the cell is encapsulated within a non-contacting silica nanocapsule. Yolk-shell encapsulation provides long-term cell viability, higher resistance against harsh environments and superior photosynthetic activiy. In addition, engineering the colloidal packing allows tunable shell-pore diameter for size-dependent permeability and introduction of new functionalities for specific molecular recognition.

Yolk-Shell structures can be easily synthetized via self-assebly of colloidal suspensions of silica nanoparticles as described in this protocol

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L. Wang et al., "Single-cell yolk-shell nanoencapsulation for long-term viability with size-dependent permeability and molecular recognition", National Science Review, vol. 8, no. 4, 2020. Available: 10.1093/nsr/nwaa097 [Accessed 26 September 2021].

KEYWORDS

Cyanobacteria, Encapsulation, Immobilization, Yolk-Shell, Nanomaterials, Biohybrids, Photosynthesis, 4C_Fuels, iGEM

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MATERIALS TEXT

- **Fresh Cyanobacterial Culture**
- LUDOX® TMA colloidal silica **Sigma**
- **Aldrich Catalog #420859**
- **Protamine Sulfate (Biochemistry Grade)**
- **PBS x1 Buffer pH = 7.4**
- **BG-11 Media (HEPES 10 mM pH = 8)**

Cells Pretreatment

10m

1 **2800 rpm, 00:10:00**

In sterility, centrifugue a suffcient ammount of exponentially growing cyanobacteria culture (20 to 100 mL) . OD_{720} should be between 0.5 to 1. It is recommended to split the culture in two different **sterile centrifugue tubes.**

2 **In sterility,** discard the supernatant and **add 1/10th of the initial volume with sterile PBS buffer.** Resuspend the cell pellet gently, gently flicking the tube or slowly pipetting up and down with a wide open pipette tip.

3 Take **1000 µl** sample from the resuspended cells and **measure the Optical Density at 720 nm** using a Spectrophotometer. **OD_{720} should be 1.3**. If **OD_{720} is greater than 1.3** , and add more **sterile PBS** till reaching the required **OD_{720} value.**

5m

4 **In Sterility,** add **1/50 part (v/v)** of the available resuspended cells volume of **5% protamine sulfate in sterile PBS.** It is recommended to **flick the tube up and down a couple of times** and incubate the cells with protamine sulfate for **00:05:00** . (No agitation required)

10m

5 **2800 rpm, 00:10:00** Centrifugue the cells and discard the supernatant.

IMPORTANT! Write the final volume of cell suspension in the tube before discarding the supernatant.

6 After centrifugation, **resuspend the cell pellet** in the **same volume of cell suspension** formerly used. Final OD_{720} should still be around 1.3

Yolk-Shell Self Assembly 22m

7 Add the **necessary ammount of Ludox(R) TMA** colloidal suspension to reach a final concentration of 1 mg/mL in the cell suspension.

$$\mathbf{V_{Ludox\text{-}TMA}} = VLudox(\mu L) = (VcellSuspension(mL)/418) * 1000$$

NOTE. Ludox TMA has a ρ = 1.23 g/mL and it's a colloidal suspension at 34% $_{wt}$, then each mL of the stock will carry 418 mg of silica nanoparticles. To know the required ammount of Ludox, just divide your cell suspension volume by 418.

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8 Incubate the suspension for **00:20:00** with mild agitation using a rocker shaker at minimum speed. 20m

Storing Yolk-Shell 10m

9 **3600 rpm, 00:04:00** After incubation, centrifugue the former suspension and **discard the supernatant**. Wash the yolk-shell pellet with fresh **BG-11 media** using the same ammount of volume used during incubation (Other media could also be used for marine cyanobacteria strains). 4m

10 Repeat the wash step , and resuspend the cells with fresh **BG-11 media** to a final **OD_{720} = 1.**

In this conditions yolk shell can be stored at room temperature and mild illumination for long periods of time.

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