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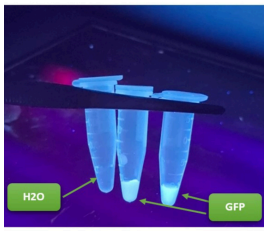
Low-Cost Sucrose Gradient Protocol for GFP Purification from Recombinant E. coli: A Visual Fluorescence-Based Method for Resource-Limited Laboratories



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

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Disclaimer

This protocol was developed and executed in a laboratory with limited infrastructure. The confirmation of GFP purification was based solely on visual fluorescence under UV light. No spectrophotometric, electrophoretic, or biochemical validation was performed to assess concentration and contaminants. As such, the results should be considered preliminary. Further studies are necessary to quantitatively assess purity, yield, and functional integrity of the extracted GFP.

Abstract

This protocol describes a simple, cost-effective method for purifying Green Fluorescent Protein (GFP) from E. coli using a sucrose gradient. It avoids toxic chemicals and expensive chromatography systems, making it accessible and environmentally friendly. The method preserves GFP's fluorescence and is suitable for applications in biomedical imaging, molecular tagging, and gene expression education laboratories.
with further improvements, it could be used in research studies.

Image Attribution

Biotechnology Engineering Department - University of Aleppo

Materials

Genetically modified E. coli BL21 (DE3) pLysS with pRSET plasmid
Sucrose (analytical grade)
Sterile distilled water
Falcon tubes (15 ml)
Microcentrifuge tubes (1.5 ml)
Ethanol (70%)
Micropipettes and tips
Centrifuge (R5810 and CF-10 models or equivalents)
UV light source

Preparation of Sucrose Gradient

7m

1 Prepare four sucrose solutions:
weigh 1, 2, 3, 4 g of sucrose

5m

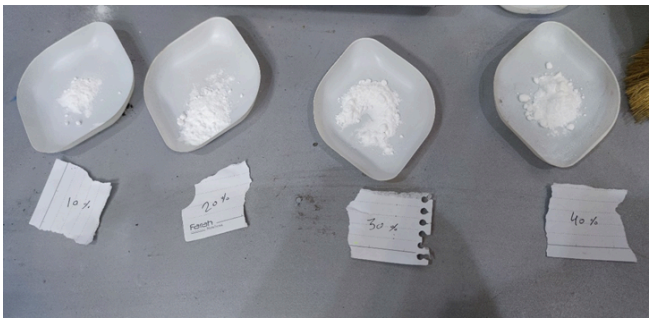

Figure (1): Prepared sucrose weights for the experiment

to be out in 10 mL sterile water in order to make
10% (Tube 1) 20% (Tube 2) 30% (Tube 3) 40% (Tube 4)

Figure (2): Sucrose powder in tubes before adding distilled water

Dissolve the appropriate sucrose amounts in sterile distilled water and mix until homogeneous.

2 Layer 2 ml of each sucrose solution sequentially into a Falcon tube using a micropipette. Begin with 40% at the bottom, followed by 30%, 20%, and finally 10% at the top. Layer gently to avoid mixing between layers.

2m

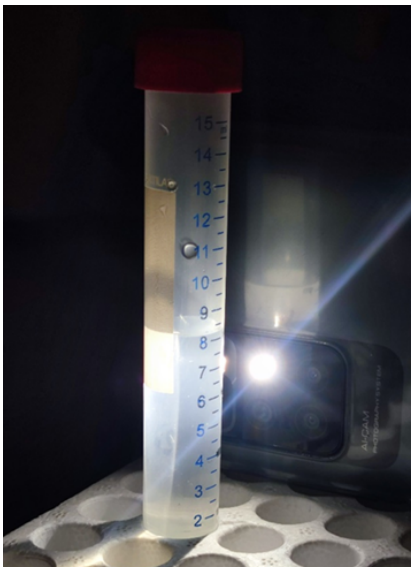

Figure (3): The gradient of sucrose layers after preparation- the layers could be visible if you spotlight a smartphone flash and move it, this can help you know if the layers interfaced. in case layers interfaces you need to redo the process.

Sample Loading and Initial Centrifugation

5m 30s

3 Centrifuge 5 ml of E. coli culture at 7000 rpm for 5 minutes in a falcon tube to pellet the bacteria. 5m

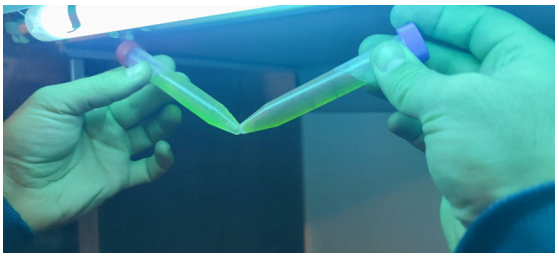

Figure (4): The bacterial culture after centrifugation. the GFP is secreted to the media.

4 Carefully collect 1 mL of the supernatant, which contains secreted proteins, and gently layer it on top of the prepared sucrose gradient. 30s

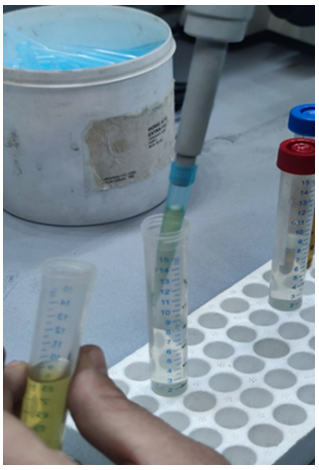

Figure (5): The process of loading the supernatant onto the sucrose gradient

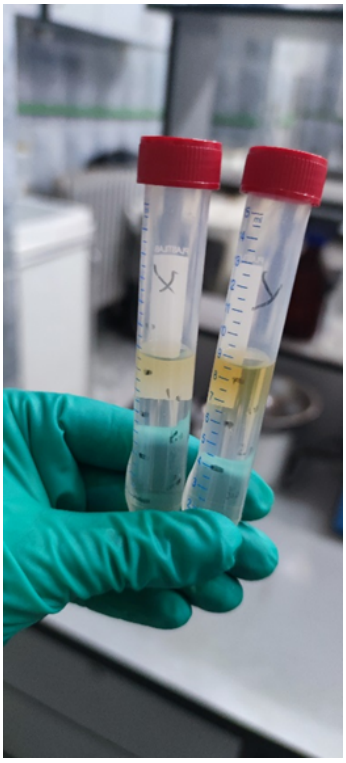

Figure (6): The sucrose gradient tube after bacterial supernatant addition

GFP Purification

32m

5 Centrifuge the gradient tube at 10000 rpm for 00:30:00 using an R5810 centrifuge. After centrifugation, observe the tube under UV light. GFP will localize between the 10- 20% sucrose layer, visible as a fluorescent band. 30m

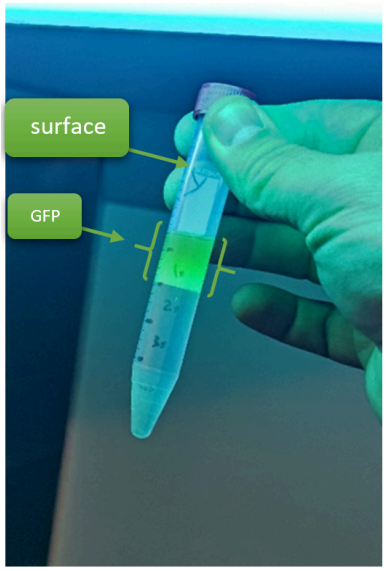

Figure (7): Protein localization and fluorescence between 10-20% sucrose gradient concentration

6 Carefully aspirate the fluorescent layer using a micropipette and transfer it to a 1.5 ml microcentrifuge tube. Centrifuge this tube at 12000 rpm for 2 minutes using a CF-10 centrifuge. 2m

Final Purification and Concentration 4m

7 Examine the pellet under UV light to confirm GFP fluorescence.

Figure (8): The purified GFP exhibited higher fluorescence intensity compared to the control water sample

8 Add 20 µL of 70% ethanol and 20 µL of distilled water to the GFP sample. Mix gently and centrifuge again at 12000 rpm for 4 minutes. 4m

9 After centrifugation, visualize the sample under UV light. The ethanol precipitation enhances fluorescence intensity, confirming successful concentration of GFP.

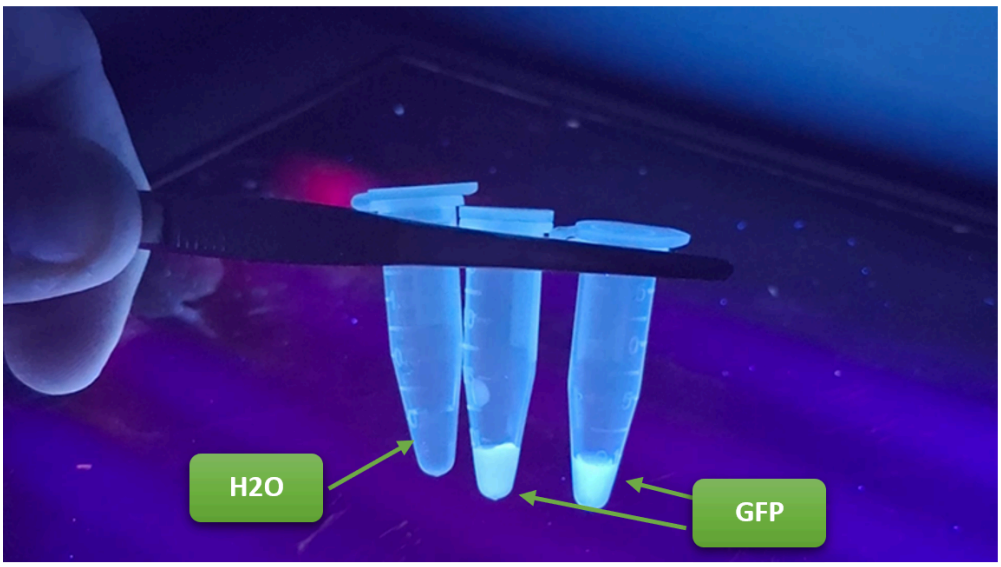

Figure (9): The ethanol-precipitated GFP sample demonstrated significantly higher fluorescence compared to the untreated control when examined under identical UV exposure conditions

Results

10 GFP is localized between 10-20% sucrose layers.
Ethanol precipitation significantly enhances fluorescence.
The final product is visibly fluorescent and suitable for downstream applications.

Protocol references

Fernández-Martínez, J., & Rout, M. P. (2016). Density gradient ultracentrifugation for the isolation of protein complexes. Cold Spring Harbor Protocols. Retrieved from https://www.ncdir.org/wp-content/uploads/2018/03/Cold-Spring-Harb-Protoc-2016-Fernandez-Martinez.pdf

Joseph shenekji, Dr. Kamar Shayah, Sidra Latfo, Roukaia Hariri, Walaa Alothman, Dr. Zaher Samman Tahhan, Mai Al-Ansary 2025. manufacturing dried cell reagents using the genetically modified bacterial strain E.coli pLysS expressing GFP protein. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.rm7vzk9j4vx1/v1**

Joseph shenekji 2024. Using Skimmed milk as a low-cost alternative to IPTG in plasmid expression induction (LacZ Promoter) . **protocols.io** **https://dx.doi.org/10.17504/protocols.io.j8nlk81z6l5r/v1**