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🌐 manufacturing dried cell reagents using the genetically modified bacterial strain E.coli pLysS expressing GFP protein

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We use this protocol and it's working

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Keywords: dried cell reagent, green fluorescent protein, bacterial suspension, successful bacterial growth, transportation of bacterial strain, bacterial culture, modified bacterial strain, adaptable preservation techniques suitable for laboratory, bacterial growth, bacterial strain, cell reagents in scientific application, impact on bacterial growth, nutrient medium with swab, cell reagent, incorporation of ampicillin, efficacy of the reagent, adaptable preservation technique, fluorescent, reagent, ampicillin, different drying, gfp protein this protocol, expressing gfp protein, preparation of nutrient agar, sterilization, fluorescence, fluorescence under specific lighting condition, supplemented petri dish, nutrient medium, protein

Abstract

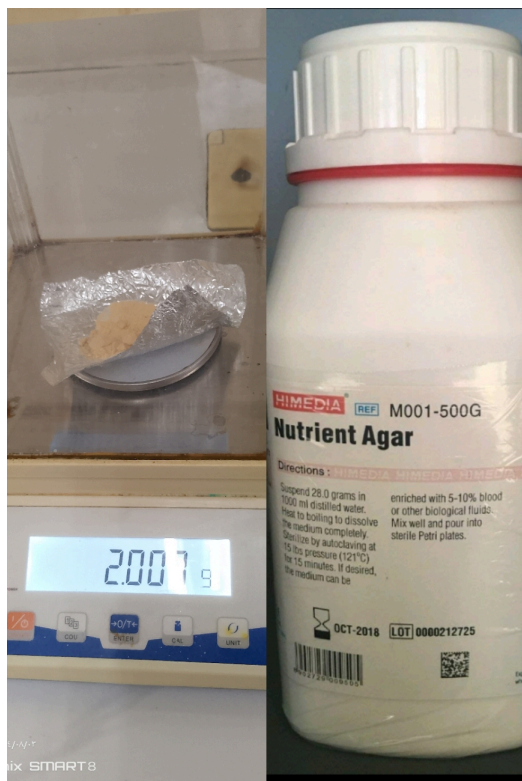
This protocol outlines the methodology for manufacturing dried cell reagents utilizing the genetically modified bacterial strain E. coli plyss, which expresses the green fluorescent protein (GFP). The process begins with the preparation of nutrient agar or LB medium, followed by sterilization and the incorporation of ampicillin as an antibiotic. Bacterial cultures are initiated by inoculating the nutrient medium with swabs from antibiotic-supplemented petri dishes and incubating them under controlled conditions. Various stimulants, including skim milk and IPTG, are introduced to assess their impact on bacterial growth, which is monitored through spectrophotometric absorbance and fluorescence under specific lighting conditions. The protocol includes centrifugation and washing steps to prepare bacterial suspensions, which are then subjected to different drying and storage conditions to evaluate the viability of the dried cell reagents. The results demonstrate successful bacterial growth, confirming the efficacy of the reagents and their potential for long-term preservation without stringent storage requirements. This method facilitates the transportation of bacterial strains across regions, reduces costs, and offers adaptable preservation techniques suitable for laboratories with limited resources. The findings highlight the feasibility of using low-cost stimulants and the sustainability of dried cell reagents in scientific applications.

Safety warnings

- ❗ 1- When performing the preservation step, it must be in a place away from moisture
- 2- Make sure to shake the nutrient media grown with strains in order to obtain the best growth

- 1 Weigh  2 g of (NA) nutrient agar or LB medium and place it in  100 mL of distilled water (water was sterilized using an autoclave for  00:20:00 at  121 °C)

20m

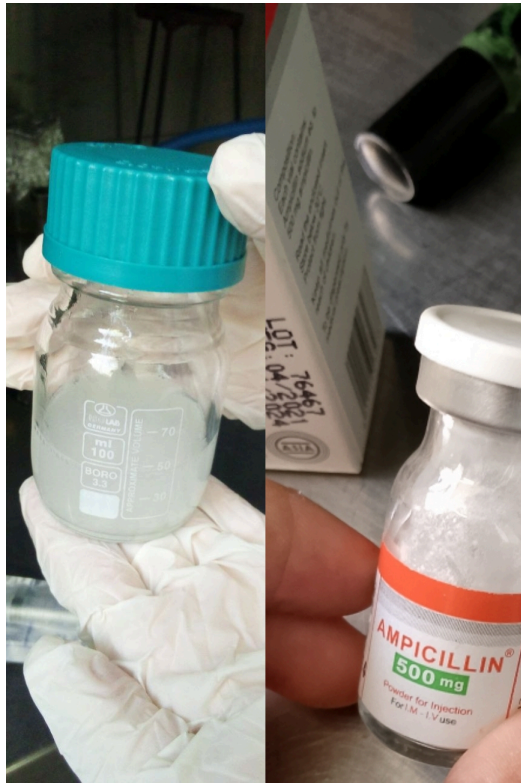


- 2 Sterilize in an autoclave at  121 °C for  00:20:00 for the nutrient medium,  10 mL tubes and  100 mL distilled water.

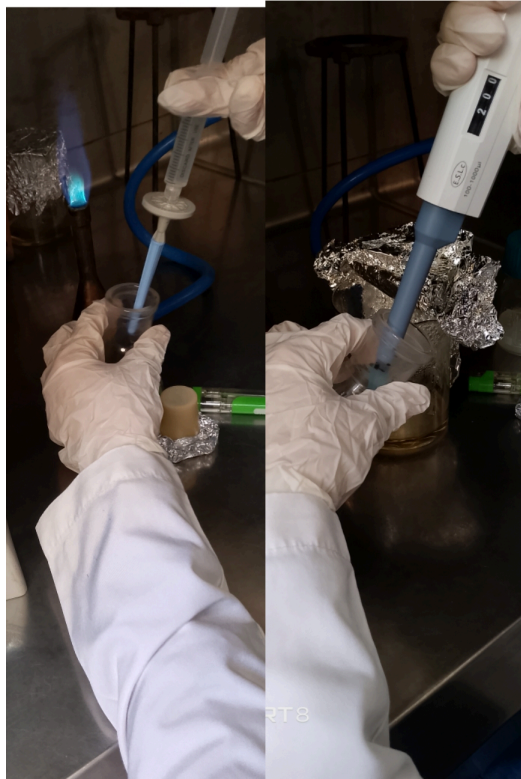
20m



- 3 Prepare the antibiotic ampicillin by taking  10 mL of sterile distilled water in step 2 and adding a capsule of ampicillin with a concentration of  500 mg and stirring until mixing is appropriate.



- 4 Take 200 μ l of the antibiotic prepared using a micropipette and add it to the previously prepared nutrient medium and mix gently.



- 5 Prepare 5 tubes with a capacity of 10 mL , each containing 6 mL of the nutrient medium to which the antibiotic has been added.
- 6 Prepare both the tubes and sterilize them by flame
- 7 Perform the bacterial culture process within the prepared tubes by taking a swab from the bacterial colonies that were grown on petri dishes containing LB medium to which the antibiotic (Ampicillin) was added and cultured within the liquid nutrient medium.



8 Incubate the nutrient media in a shaking bacterial incubator at a temperature of

🌡️ 37 °C Celsius for ⌚ 04:00:00

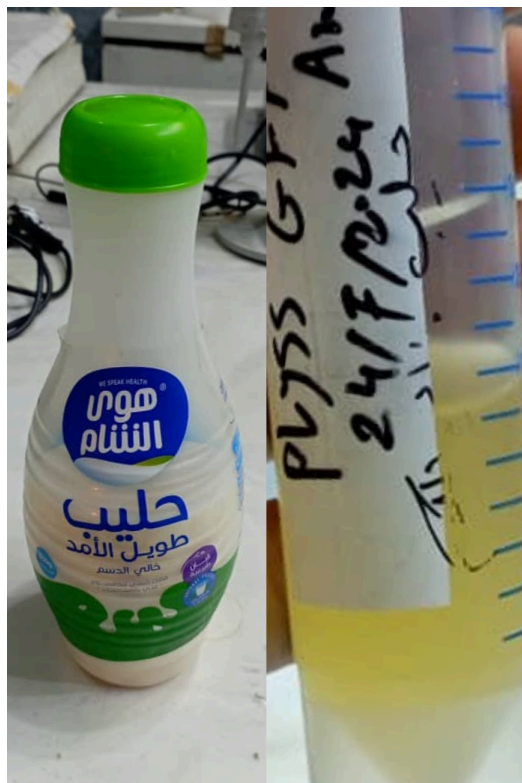
4h

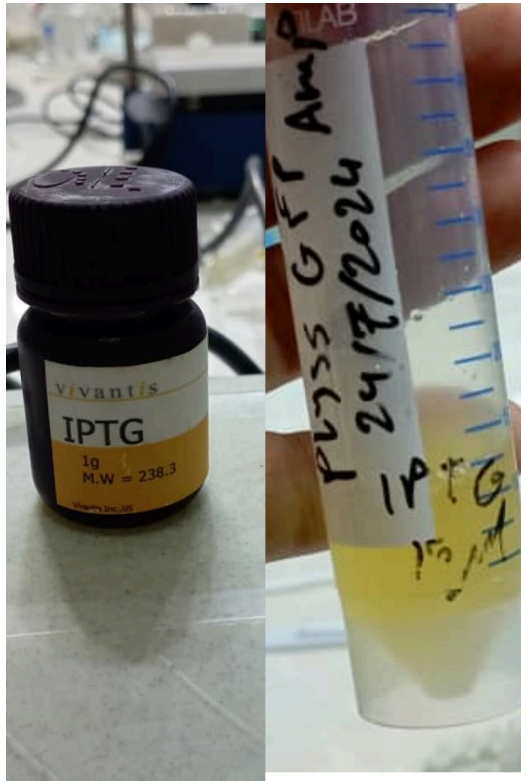


9 After 4 hours of incubating the media, add (stimulant):

Add  200 μL of skimmed milk using a micropipette tube, or you can use

[M] 0.1 Mass Percent IPTG  15 μL



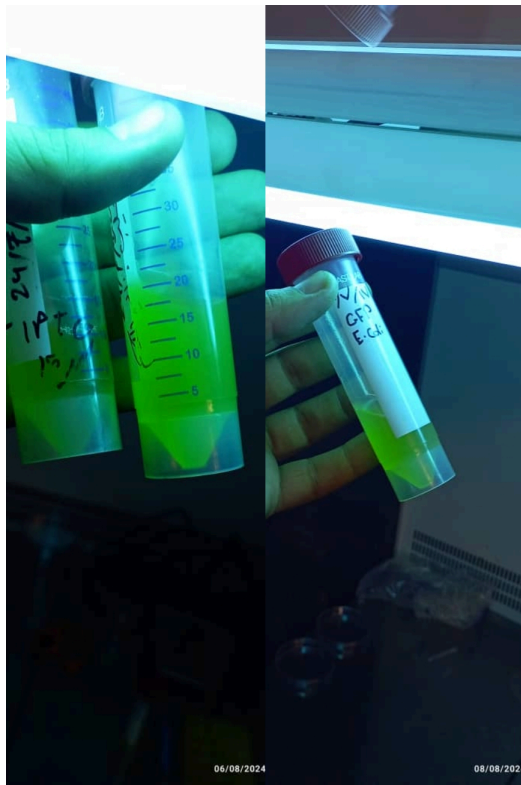


- 10 Incubate the media to which stimuli were added in a shaking bacterial incubator at  37 °C for  24:00:00 .
- 11 Take  1 mL of the incubated media (step 10) to measure the absorbance on a spectrophotometer at A600 wavelength to confirm bacterial growth

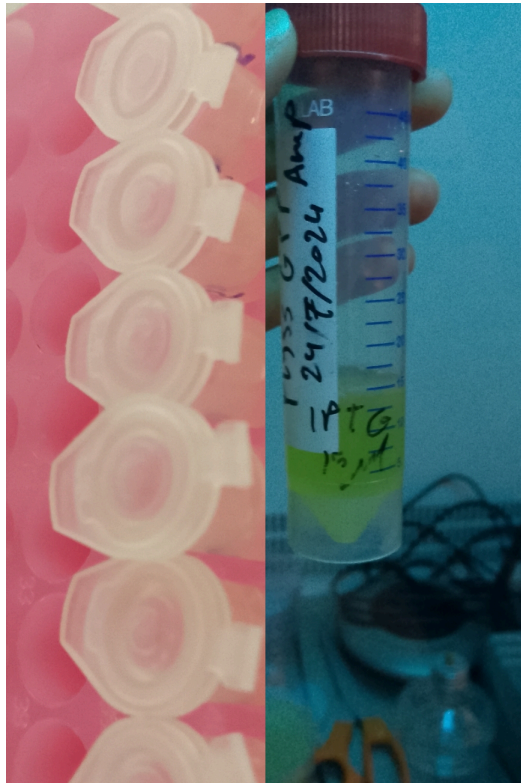
1d



- 12 Visually detect bacterial growth using blue or UV light to see the fluorescence of the green GFP protein



- 13 Take 200 μ l from the tube containing the IPTG stimuli and place it in 5 small tubes of 1.5 ml capacity respectively.



- 14 Perform centrifugation at  9000 rpm so that the nutrient medium is at the top and the bacterial suspension is at the bottom.



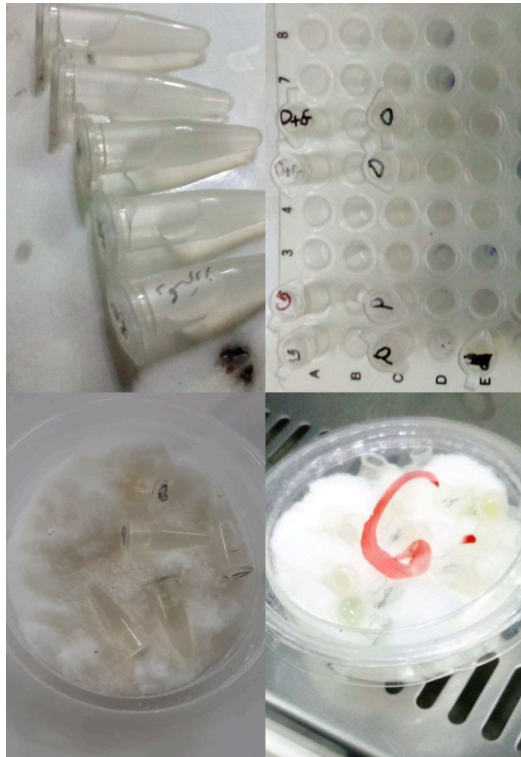
- 15 Discard the floating solution and add  1 mL of cold PBS solution with a concentration of 1X, then perform the centrifugation step again and discard the floating solution while preserving the bacterial suspension.



- 16 Add different washing solutions to the previous tubes containing the bacterial suspension as follows:



- The first tube: with a capacity of 1.5 ml, add 250 microliters of glycerol and 1ml of cold PBS solution with a concentration of 1X (glycerol concentration 25%).
 - The second tube: with a capacity of 1.5 ml, add 0.05 g of dextrose with 1 ml of cold PBS solution with a concentration of 1X (dextrose concentration 50%).
 - The other three tubes: 1 ml of cold PBS solution with a concentration of 1X is added.
- 17- Prepare 8 tubes with a capacity of 0.2 ml and fill them as follows:



- * Two tubes are added to 200 μ l of bacterial suspension and dextrose.
- * Two tubes are added to 200 μ l of bacterial suspension and glycerol.
- * Two tubes are added to 100 μ l of bacterial suspension and dextrose and 100 μ l of bacterial suspension and glycerol.
- * Two tubes are added to 200 μ l of cold PBS solution with a concentration of 1X.
- * A control tube with the same capacity contains only culture medium.

17 Place the tubes containing the preservation solutions in a tightly sealed container containing silica granules (the purpose of the silica granules is to absorb moisture) while leaving the tube caps open and closing the container tightly.

18 Place some of the tubes prepared in step 17 under different drying conditions as follows:

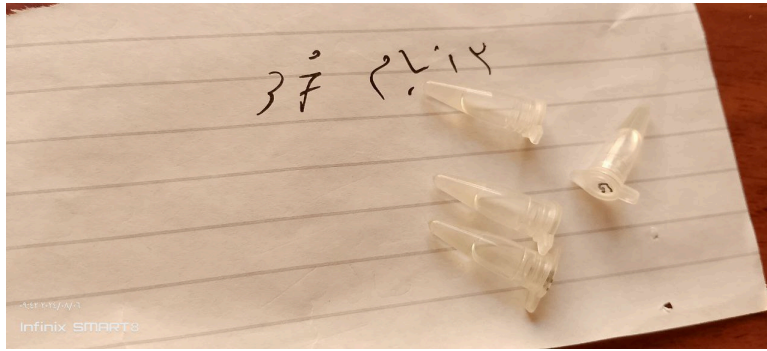
- * In the incubator at a temperature of 37 degrees Celsius.
- * Others are placed at room temperature for 48:00:00 for drying.

2d

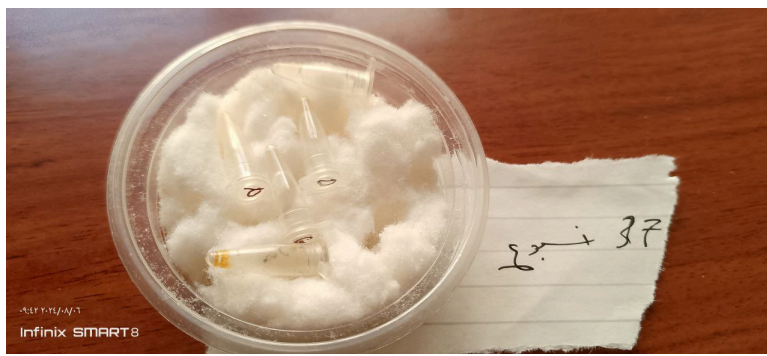
19 Place the dried tubes under different storage conditions and for different periods of time as follows:

- * In the incubator at a temperature of 37 °C Celsius for 72:00:00

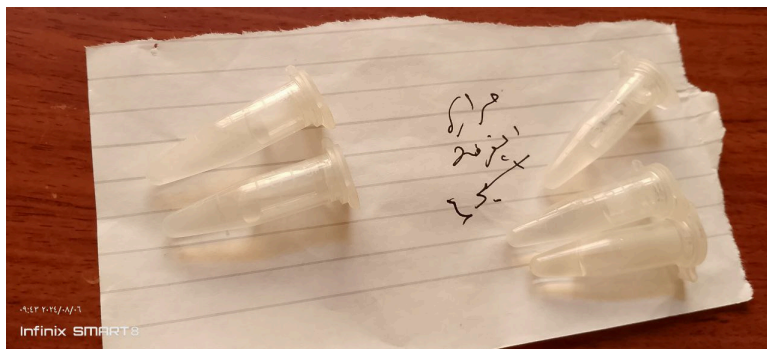
1w 3d



* In the incubator at a temperature of 37 degrees Celsius for 7 days



* In the laboratory room at room temperature for 🕒 168:00:00 .



20 After the storage period has elapsed, the dried cell reagents are rehydrated and cultured to ensure the effectiveness of the dried cell reagents and the ability of the bacteria to grow again as follows:

1d

Rehydrate by adding 30 μ l distilled water (prepared in step 2).

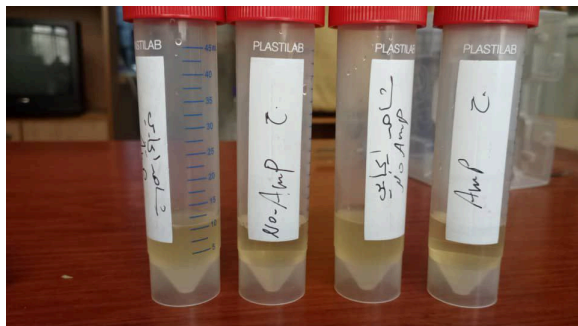
Prepare NA nutrient medium with the antibiotic ampicillin (steps 1, 3).

Pour the prepared nutrient medium into sterile petri dishes and tubes in an autoclave at 121°C for 20 minutes.

Take 15 μ l of the rehydrated cell reagents and culture them on petri dishes containing NA medium (prepared in step 17-3)

Incubate in a shaking bacterial incubator at 37°C for 24:00:00 .

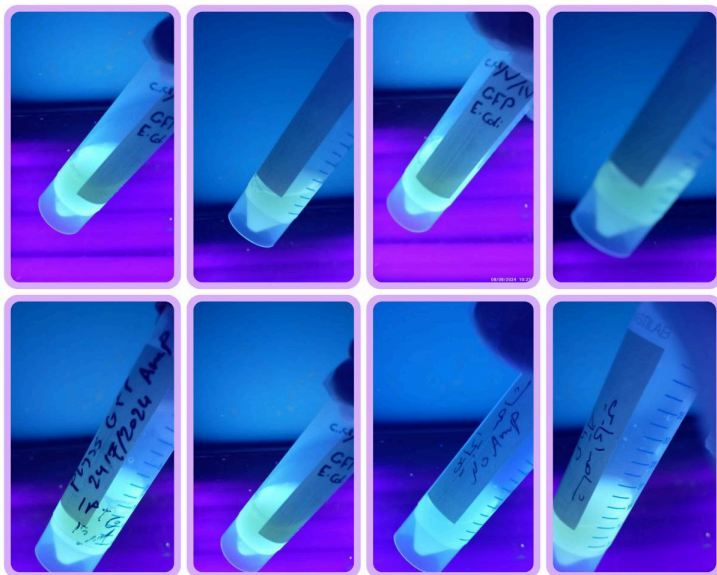
Positive control tube containing the bacterial strain



21 Results:

- 1- Bacterial growth was obtained when culturing dried cell reagents, thus proving the effectiveness of these reagents.
- 2- The possibility of manufacturing cell reagents and preserving them for a period of time without the need for specific storage conditions
- 3- Ease of transporting bacterial colonies and proteins between governorates and countries.
- 4- Reducing the cost of transporting and preserving bacterial strains
- 5- Using different stimulants and preservation solutions to manufacture cell reagents.
- 6-Using skim milk as a stimulant instead of IPTG.
- 7-Store cell reagents for one week at room temperature.
- 8- Propagation and growth of bacteria producing fluorescent GFP protein.
- 9- Using blue light instead of ultraviolet rays to visually monitor the expression of this protein.
- 10- Using the antibiotic available in regular pharmacies gave good effectiveness, which saves the costs of pure solutions used for bacterial culture.
- 11- Providing 6 methods for preserving bacteria that can be used according to the capabilities of laboratories in conditions where there is no electricity or when transporting bacteria from one place to another that takes several days to reach.
- 12- Cellular reagents can be used to preserve genetically modified bacterial strains.
- 13- Low-cost catalysts can be used as alternatives to expensive genetic stimulation materials such as skim milk.
- 14- Cellular reagents are low-cost and more sustainable compared to pure proteins.
- 15- Cellular reagents can be produced by all laboratories with limited capabilities.





Protocol references

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