

# Lyophilization

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## Introduction

This protocol has been conceived following three different lyophilization instructions:

- Phillips, T. (2019). How to Freeze-Dry a Bacterial Culture (Lyophilization). *ThoughtCo.*, Science, Technology, and Math. <https://www.thoughtco.com/how-to-freeze-dry-a-bacterial-culture-lyophilization-375685>
- Survival of *Escherichia coli* in Four Lyophilization Solutions. *OPSDiagnostics*. [https://opsdiagnostics.com/applications/lyophilization/ecoli\\_lyophilization\\_stability.html](https://opsdiagnostics.com/applications/lyophilization/ecoli_lyophilization_stability.html)
- XMU-China iGEM Team (2017). Freeze-Dry. *iGEM*. <http://2017.igem.org/Team:XMU-China/Freeze-Dry>

## Materials

- › Bacterial colonies (samples)
- › 10% sucrose freeze drying buffer
- › Lyophilization vials
- › Split stoppers
- › Lyophilization devices

## Procedure

1. Overnight culture at 37°C on LB Medium with shaking (OD≈1,15).
2. Centrifugation of 1mL of the cell suspension (12000r, 2min) + Supernatant discarded .
3. Resuspension of the bacterial pellets in 500µL of 10% sucrose freeze drying Buffer sterilized.
4. Transfer into lyophilization vials
5. Capping with split stoppers
6. Freezing for 2 hours at -40°C.
7. Primary drying for 16 hours at -25°C
8. Secondary drying for 3 hours at 25°C.
9. Sealing of vials under vacuum.
10. Vials storage at 4°C.

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