



Oct 21, 2021

Glycerol Stock Preparation for *S. elongatus*

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dx.doi.org/10.17504/protocols.io.bzb4p2qw

iGEM IISER Pune India 2021

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Bacterial cultures in the form of agar plates or liquid cultures are not suitable for long term storage at low temperatures such as 4 degrees. Long-term storage of bacteria is best at temperatures around -80 degrees. However, agar plates and liquid cultures cannot be stored at such temperatures, as the water crystallizes in and around the bacterial cells, rupturing and killing them. Glycerol or DMSO (anti-freezing agents) is added to the media to store samples at such temperatures.

DOI

dx.doi.org/10.17504/protocols.io.bzb4p2qw

Akashdutta 2021. Glycerol Stock Preparation for *S. elongatus*. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bzb4p2qw>



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1 To make n stocks, take an aliquot of the *S. elongatus* culture such that the OD times the volume is at least 5n.

2 Centrifuge this aliquot in a falcon at **5000 rpm, 25°C, 00:07:00**

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- 3 Discard the supernatant.
- 4 Resuspend the pellet in the minimum amount of BG11 medium required to do so.
- 5 Add n mL of 25% glycerol and mix well.
- 6 Separate equally into n cryovial tubes.
- 7 Store in the stocks in -80°C .