



Jun 25, 2020

# Making Freezing Solution

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## ABSTRACT

The freezing solution can be made in advance and stored in sterile containers at room temperature till needed.

## DOI

[dx.doi.org/10.17504/protocols.io.bhwgj7bw](https://dx.doi.org/10.17504/protocols.io.bhwgj7bw)

## PROTOCOL CITATION

Priota Islam 2020. Making Freezing Solution. **protocols.io**  
[dx.doi.org/10.17504/protocols.io.bhwgj7bw](https://dx.doi.org/10.17504/protocols.io.bhwgj7bw)

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## CREATED

Jun 25, 2020

## LAST MODIFIED

Jun 25, 2020

## PROTOCOL INTEGER ID

38568

## Steps

- 1 Book the autoclave beforehand
- 2 Make 0.05M  $K_2HPO_4$  by dissolving 8.71g of  $K_2HPO_4$  in 1L of DIW
- 3 Make 0.05M  $KH_2PO_4$  by dissolving 6.80g of  $KH_2PO_4$  in 1L of DIW
- 4 Make S Buffer solution by adding the following:  
129 ml 0.05 M  $K_2HPO_4$   
871 ml 0.05 M  $KH_2PO_4$   
5.85 g NaCl
- 5 Make the freezing solution by adding the S buffer to 30% glycerol (v/v):

Just add 700ml of S buffer to 300ml of glycerol to get the desired concentration

- 6 Autoclave the mixture. (Aliquoting first is not advised due to handling issues upon adding glycerol)
- 7 After autoclaving, aliquot into sterile 200ml bottles labelled with the name and date of the solution prepared.

#### Notes

- 8 Before using the freezing solution always check the bottle for any growth/contaminants
- 9 Discard the bottle if you see any contaminants and open a new one
- 10 Aliquot into falcon tubes from 200ml bottles to reduce contamination. Discard the tube after freezing do not put the contents back to the 200ml bottle!