Nov 25, 2020

Enterococcus faecalis protoplast generation

Elizabeth Fozo[1]
[1]In-house protocol

1 Works for me This protocol is published without a DOI.

Eadewunm

PROTOCOL CITATION

Elizabeth Fozo 2020. Enterococcus faecalis protoplast generation. **protocols.io** https://protocols.io/view/enterococcus-faecalis-protoplast-generation-bp24mqgw

LICENSE

CREATED

Nov 24, 2020

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PROTOCOL INTEGER ID

44860

MATERIALS TEXT

Required media
- BHI
- Isotonic solution – 20% sucrose, 0.145M NaCl, 50mM Tris-HCl

DISCLAIMER:

BEFORE STARTING

Required media
- BHI
- Isotonic solution – 20% sucrose, 0.145M NaCl, 50mM Tris-HCl

Protocol

1 Grow OG1RF in BHI + condition until OD_{600} 0.3. This works for as little as 10mls of culture.

Growing in 2% glycine will reduce “gluing” of cells, but massively increase generation time.

2 Spin cells down and resuspend in half volume isotonic solution.

3 Add 2mg/ml lysozyme and incubate for 60 minutes. More time may be required.

4 Check for wall removal using Gram stain. (Do not move on until protoplast confirmation)

5 Spin cells down and wash with isotonic solution, resuspending again in isotonic solution in half the volume of the original culture.

When resuspending cells after lysozyme treatment, pipette mix and refrain from over-vortexing as this will kill protoplast cells.