OCT 26, 2018

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.u3heyj6

Protocol Citation: Josef Hoff 2018. Preparation of electrocompetent cells. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.u3heyj6>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Oct 26, 2018

Last Modified: Oct 26, 2018

PROTOCOL integer ID:
 17225

🌐 Preparation of electrocompetent cells

Josef Hoff¹

¹Phillips University

Josef Hoff

ABSTRACT

Preparation of electrocompetent cells

Weinstock paper:

[Matthew T Weinstock](#), [Eric D Heseck](#), [Christopher M Wilson](#), [Daniel G Gibson](#)

Vibrio natriegens as a fast-growing host for molecular biology

Nature Methods volume 13, pages 849–851 (2016)

To prepare the day before:

- LB I media +V2 salts (204 mM NaCl, 4.2 mM KCl, 23.14mM MgCl₂)
- electroporation buffer (680 mM sucrose, 7 mM K₂HPO₄, [pH 7](#)) (sterile filtrated)
- over night culture of V.n from the cryo-stock in LBI + v2 salts (37 °C ; at 200 r.p.m)

Preparing culture :

Depending on how many aliquds you want to have, incubate media with your overnight culture for a starting OD of 0.05.

The culture is grown at 37 °C in a baffled flask, shaking at 200 r.p.m. until an OD600 between 0.5 to 0.8 is reached.

*be careful when they reach an OD near 0.1, *V. natriegensis* very fast growing, so start measuring in shorter time periods.

Prechill the electroporation buffer

Washing:

From here on try always to keep your culture on ice.

- The culture is then put on ice for 15 min.
 (the original Protocol sais to directly fill them into your prechilled centrifugatin containments)
- The cells are pelleted at 3000x g. for 20 min at 4 °C.
- The supernatant is carefully decanted and the cell pellets are gently suspended in

10mL

of **chilled** electroporation buffer.

-The suspensions are transferred to a **chilled** 50mL falcon tube and the tube is filled top

with additional **chilled** electroporation buffer (50mL) and inverted several times.

-The cells are centrifuged down at 3000x g for 15 min at 4 °C.

-The wash is repeated two times for a total of three washes.

Aliquotation:

After the final wash, the supernant is carefully decanted the cells are gently resuspended in residual electroporation buffer.

Measure the OD in a 1/20 dilution against electroporation buffer.

The volume is adjusted with additional electroporation buffer to bring the final OD600 to 16.

The Cells are aliquoted (80µL) into chilled 1.5µL centrifugation tubes, directly frozen in liquid nitrogen and stored at -80 °C until use.

ATTACHMENTS

[Preparation of
electrocompetent
cells.pdf](#)