Preparation of Chemical Competent Cells

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Version: 1.0 - Print Version (.PDF)

This is in my opinion the best protocol for chemical competent bacteria. The bacteria can get as competent as electrocompetent cells if you are good.

Prepare first:

- 1 L SOB medium
- 200 ml Inoue solution
- 20 ml DMSO of highest quality!
- 4x 1 L Erlenmeyer flasks autoclaved/sterile
- 4x 250 ml centrifuge tubes for Beckam rotor J14 autoclaved/sterile
- 5 ml pipet tips
- At least 500x 0.5 ml eppendorf tubes autoclaved/sterile
- Liquid nitrogen
- Required: Centrifuge Beckam with rotor J14 or similar

Work very sterile because we do not use any antibiotics!

1. Reserve 15 ml SOB in a small bottle at 4 °C
2. Inoculate 1 L SOB Medium with a starter culture and mix well
3. Spread to 4x 1 L Erlenmeyer flasks
4. Incubate at 18°C or room temerature shaking 200 rpm over night
5. Measure OD 600 in the morning of all four flasks
6. If the first flask reaches OD 0.45 put all flasks for 10 min on an ice-water bath
7. Pellet at 2500 G for 10 min at 4 °C - Cold Room!
8. Resuspend in 4x 80 ml cold Inoue solution 4 °C
9. Pellet at 2500 G for 10 min at 4 °C
10. Resuspend in 4x 20 ml cold Inoue solution 4 °C
11. Pool the 4 aliquots and add 6 ml of warm DMSO
12. Incubate for 10 min
13. Make 200 µl aliquots in 0.5 ml eppendorf tubes
14. Freeze in liquid Nitrogen and store at -80 °C

Buffers:

SOB medium
5 g NaCl
20 g trypton
5 g yeast extract
2.5 ml 1 M KCl
add up to 1 litre water and autoclave

Inoue solution
10.9 g $MnCl_2$ - **Manganesecloride!**
2.2 g $CaCl_2$
18.7 g KCl
20 ml 0.5 M PIPES solution
add 1 L water and filter-sterilize

0.5 M PIPES solution
PIPES = piperazine-1,2-bis[2-ethanesulfonic acid]
Dissolve 15.1 g PIPES in 80 ml water
by trowing in KOH until the solution clears up.
The pH should be close to the desired pH 6.7.
If it is too low, add KOH, if it is too high, add HCl.
Add water until 100 ml and aliquot in 10 ml tubes.
Store at -20°C.

Commented Protocol:

1. Reserve 15 ml SOB in a small bottle at 4 °C

We will need SOB without bacteria as a blank sample to measure the OD tomorrow.

2. Inoculate 1 L SOB Medium with a starter culture and mix well

As a starter culture you can use an overnight grown 3 ml culture which was picked from a single colony. I prefer to use the Starter Glycerol Stocks.

3. Spread to 4x 1 L Erlenmeyer flasks

Each Erlenmeyer should be filled with not more than 250 ml. To grow bacteria flasks should be never filled with more than a quarter of it's volume or the aeration is limiting.

4. Incubate at 18°C or room temerature shaking 200 rpm over night

5. Measure OD 600 in the morning of all four flasks

Use the 15 ml SOB from the day before to blank. Measure all 45 min till the desired OD is reached.

6. If the first flask reaches OD 0.45 put all flasks for 10 min on an ice-water bath

This is the step which limits quality most. Bacteria are most competent at OD 0.4-0.5 and 0.9. Because it is very difficult to catch them at OD 0.9 every protocol uses OD 0.4-0.5. If the bacteria are over OD 0.5 the competence will be reduced.

The original protocol recommends to harvest the one flask which reaches OD 0.55 first and trash all the other cultures. If highest competent cells are what you are after, that is an option.

7. Pellet at 2500 G for 10 min at 4 °C - Cold Room!

Never let the bacteria warm up again! If you can, work in a cold room on ice. The quality of the competent cells will compensate for the uncomfortable time.

From now on it is not necessary to worry about sterility so much. If you get a contamination, it will result in one or two colonies on a plate, so nothing dramatic.

8. Resuspend in 4x 80 ml cold Inoue solution 4 °C

9. Pellet at 2500 G for 10 min at 4 °C

10. Resuspend in 4x 20 ml cold Inoue solution 4 °C

11. Pool the 4 aliquots and add 6 ml of warm DMSO

DMSO gets solid at around 4 °C, that's why we use it warm.

12. Incubate for 10 min

Let the DMSO diffuse into the cells.

13. Make 200 µl aliquots in 0.5 ml eppendorf tubes

Cool the eppis! I put the eppis on ice and fill them fast with a multi-pipet. Be fast and let a colleague help you. One fills the tubes the other one closes them and throws them into liquid nitrogen.

14. Freeze in liquid Nitrogen and store at -80 °C

In -80 °C the cells will stay good at least half a year. Test them after production and retest them if you are not sure if they are still OK. See the transformation protocol for details. At best you can reach 1-3 x 10^8 col / µg plasmid.

Known Issues:

- Work fast, clean and cold - you will get good cells. The more practice you get the better the cells will be. If you are not happy with the results, just repeat it and they will be good.

References and Comments:

This is a common protocol as it can be found in Maneatis or other protocol books. It is based on the article of Inoue et al. 1990. I rewrote the protocol to stress some crucial points and to make it very clear. I made my competent cells with this protocol for over 3 years with constant good results.

How to cite this page in publications:

This document can be cited like this:

Untergasser A. “Preparation of Chemical Competent Cells” *Untergasser's Lab*. Winter 2008. (include here the date when you accessed these page).
<http://www.untergasser.de/lab/protocols/competent_cells_chemical_v1_0.htm>.

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