SOB

SOB stands for super optimal broth.

SOB Medium: Used in growing bacteria for preparing chemically competent cells

Ingredients

- 0.5% (w/v) yeast extract
- 2% (w/v) tryptone
- 10 mM NaCl
- 2.5 mM KCl
- 20 mM $MgSO_4$

Per liter:

- 5 g yeast extract
- 20 g tryptone
- 0.584 g NaCl
- 0.186 g KCl
- 2.4 g $MgSO_4$

Note: Some formulations of SOB use 10 mM $MgCl_2$ and 10 mM $MgSO_4$ instead of 20 mM $MgSO_4$.

SOB medium is also available dry premixed from Difco, 0443-17.

Adjust to pH 7.5 prior to use. This requires approximately 25 ml of 1M NaOH per liter.

SOC

In addition to the SOB contents, SOC also contains 20Mm glucose (3.603g).

BUFFERS

1. **CCMB80 BUFFER**

- 10mM KOAc pH 7.0 (10ml of a 1M stock/L)
- 80mM $CaCl_2.2H_20$ (11.8 g/L)
- 20mM $MnCl_2.4H_20$ (4.0 g/L)
- 10mM $MgCl_2.6H_20$ (2.0 g/L)
- 10% glycerol (100 ml/L)
- Adjust pH down to 6.4 using 0.1N NaOH if necessary.
- Sterile filter and store at 4C

Note: Slight dark precipitate may appear. However, it will not affect its function.

2. **TAE BUFFER (pH 8.3)**

- Tris base – 48.4g/l.
- Glacial acetic acid 11.4ml/l.
- 0.5M EDTA – 20ml. (3.72g for 20ml)

The volume is made up to 1l using distilled water.

3. **TE BUFFER (pH 8)**

- Trise base – 1.214g/10ml
- 0.5M EDTA – 2ml (0.372g for 2ml).
- HCl – To adjust the pH to 8.

The volume is made up to 1l using distilled water.

COMPETENT CELLS PREPARATION

- Inoculate a loop full of culture in 5ml of LB broth and incubate it in a shaking incubator at 200rpm for 24hrs.
- Inoculate 1ml of the overnight culture in 250ml of LB broth.
- Incubate the broth containing the culture in a shaking incubator at 200rpm at 37° C until the OD_{610} reaches 0.3-0.6.
- Freeze the culture at 4° C for 30minutes to arrest the metabolism.
- Then aliquot the culture equally into 15ml falcon tubes.
- Centrifuge the tubes at 6000rpm for 10minutes. Discard the supernatant and collect the pellet.
- Add 4ml of chilled CCMB80 to the pellet and resuspend them gently using a pipette.
- Centrifuge the tubes at 6000rpm for 10minutes. Discard the supernatant and collect the pellet.
- Resuspensd once again using 2ml of chilled CCMB80.
- Centrifuge the tubes at 6000rpm for 10minutes. Discard the supernatant and collect the pellet.
- Resuspend the pellets using 1ml of chilled CCMB80.
- Centrifuge the tubes at 6000rpm for 10minutes. Discard the supernatant and collect the pellet.
- Resuspend the pellet in 100µl of chilled CCMB80 and combine the cell masses in all the tubes.
- Thus competent cells are prepared using CCMB80 buffer.

Note: Resuspension and centrifugation processes must be performed at 4° C to produce efficient competent cells.

TRANSFORMATION

- Add 50µl of competent cell culture to pre-chilled 1.5ml vial.
- Add 1µl of given plasmid DNA to it and incubate in ice for 30minutes.
- Heat-shock the cells by placing the vials in a water bath at 42° C for 90seconds.
- Place the vials in ice for 10minutes
- Add 600µl of LB broth and incubate the culture in a shaking incubator at 200rpm and 37° C for 2hours.
- Plate the culture on chloramphenicol plates and incubate the plates at 37° C

Note: Colonies will be formed only after 24-36hrs of final incubation.