



PSB-D protoplasting

Buffers

Digestion buffer 50ml - filter sterilized

- 3.6 g D-mannitol (0.4 M)
- 0.05 g KCl (20 mM)
- 0.23 g MES monohydrate (20 mM)
- 0.75 g cellulase Y-C Kyowa (1.5%)
- 0.2 g macerozyme R-40 Duchefa (0.4%)

Dissolve on a magnetic stirrer

Wash buffer 1L - filter sterilized

- 72.9 g D-mannitol (0.4 M)
 - 0.36 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2.5 mM)
 - 0.2 g MES monohydrate (1 mM)
- pH 5.7

Resuspension buffer 100ml - filter sterilized

- 7.3 g D-mannitol (0.4 M)
 - 0.3 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (15 mM)
 - 0.1 g MES monohydrate (5 mM)
- pH 5.6

Materials

- Big Petri dishes diameter 12 cm
- Shake platform
- Microscope to check your protoplasts
- Fuchs-Rosenthal cell counting chamber
- pipetboy

Protocol

1. Take 20 ml of a 2-day old subcultured PSB-D cell culture
2. Spin down 5 min 800 rpm
3. Remove supernatant carefully with 25 ml pipette
4. Add 25 ml digestion buffer
5. Carefully mix and pour in a Petri dish
6. Start shaking the plates on the platform, while covered with aluminum foil
7. Once an hour, check a drop of your cells under the microscope
8. Wait until the cells become separate, spherical cells. Usually after 4 to 5 hours
9. Transfer the solution from Petri dish to 50 ml falcon tubes with 25 ml pipette. Be careful!
10. Spin down 5' 800 rpm
11. Remove supernatant by pouring
12. Wash 2x with 20 ml wash buffer
13. Resuspend the protoplasts in 10 ml resuspension buffer
14. If necessary, estimate the concentration of the protoplasts in a counting chamber
15. Keep on ice or refrigerated



Expected results: After 4 to 5 hours your protoplasts should look like this (nicely, separated, spherical cells)