Cell Preparation for Electroporation of Aurantiochytrium using Salt and Sucrose solutions

Mariana Rius

Abstract

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Protocol

Harvest cells from culture

Step 1.

Harvest 2x 1.5 ml of cells from a culture beyond three days maturity. Centrifuge in microcentrifuge tubes at 4 C, 5 min at 12000 g.

Rinse with salt solution

Step 2.

Discard supernatant and resuspend pellet in 1 ml of salt solution (10 mM KCl, 10 mM NaCl, and 3 mM CaCl2). Centrifuge at 4 C, 5 min at 12000 g.

Rinse with sucrose solution

Step 3.

Discard supernatant and resuspend pellet in 1 ml of sucrose solution (50 mM sucrose). Centrifuge at 4 C, 5 min at 12000 g.

Repeat rinse with salt solutio

Step 4.

Discard supernatant and resuspend pellet in 1 ml of salt solution (10 mM KCl, 10 m M NaCl, and 3 mM CaCl2). Centrifuge at 4 C, 5 min at 12000 g.

Resuspend in an adequate volume of 50 mM sucrose solution

Step 5.

Discard supernatant and resuspend pellet in an adequate volume of 50 mM sucrose solution. Cell concentration should be around 4x10^7. Keep cells on ice.

NOTES

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Completion of protocol results in 15% survivorship.