Plant cell protoplast isolation

Steven Burgess

Abstract

A protocol for isolation of protoplast from plant cells adapted from Yoo et al. 2007 (http://www.nature.com/nprot/journal/v2/n7/full/nprot.2007.199.htm). Procedure was optimized for use on *Arabidopsis thaliana* tissue.

Citation: Steven Burgess Plant cell protoplast isolation. **protocols.io**
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Protocol

Step 1.

Grow plants of *Arabidopsis thaliana* (ecotype Col0) on a 3:1 (v/v) compost to vermiculite mixture at 20°C, under short day conditions (12h light:12h dark) and low light intensities (50-100µE m^{-2} s^{-1}).

Step 2.

Prepare fresh enzyme solution and transfer to glass petridish.

PROTOCOL

. Protoplast Isolation - Enzyme Buffer

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Step 2.1.

500mM MES, pH 5.6

AMOUNT
1 ml Additional info:

NOTES
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Final concentration is 1.5% (w/v)

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Final concentration is 10mM. Yoo et al. 2007 mention that MES is preheated to 70°C for 3-5 minutes prior to addition of the enzyme powder.

Step 2.2.

Mannitol

AMOUNT
5 g Additional info:

NOTES
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Final Concentration is 0.3% (w/v)

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Final concentration is 1.5% (w/v)

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Final concentration is 0.6M

Step 2.3.

1M Potassium Chloride

KCl

AMOUNT
1 ml Additional info:

NOTES
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Final Concentration is 0.3% (w/v)

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Final concentration is 1.5% (w/v)

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Final concentration is 20µM

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Final concentration is 0.6M

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Final concentration is 10mM. Yoo et al. 2007 mention that MES is preheated to 70°C for 3-5 minutes prior to addition of the enzyme powder.

Step 2.4.

Add dH_2O up to 50mL

NOTES

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Final Concentration is 0.3% (w/v)

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Final concentration is 1.5% (w/v)

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Final concentration is 20µM

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Final concentration is 0.6M

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Final concentration is 10mM. Yoo et al. 2007 mention that MES is preheated to 70°C for 3-5 minutes prior to addition of the enzyme powder.

Step 2.5.

Cellulase R10

NOTES

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Final Concentration is 0.3% (w/v)

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Final concentration is 1.5% (w/v)

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Final concentration is 20µM

Step 2.6.

Macerozyme R10

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Final Concentration is 0.3% (w/v)

Step 2.7.

Heat the enzyme solution at 55°C for 10 min, then allow to cool to room temperature.

Step 2.8.

1M Calcium Chloride

$CaCl_2$

AMOUNT
50 µl Additional info:

Step 2.9.

Step 2.10.

Filter final solution through a 0.45-µm syringe filter.

Step 3.

Remove 20-40 leaves from 4 week old Arabidopsis thaliana rosettes.

Step 4.

Pile up 5-10 leaves at a time, cut into thin (2mm) strips across the leaf using a sharp scapel blade dipped into ethanol. Place strips into enzyme solution. Repeat until all leaves have been cut.

Step 5.

Vacuum infiltrate the enzyme solution.

DURATION
00:30:00

Step 6.

Leave cells in the petri dish at 20°C without shaking.

DURATION
04:00:00

Step 7.

Twice filter cells and enzyme solution through a 125µm nylon mesh to remove residual debris into a pointed bottom, glass test tube.

Step 8.

Leave cells to settle to the bottom of the tube.

DURATION
00:30:00

Step 9.

Carefully aspirate out the liquid solution leaving the cell pellet intact.

Step 10.

Add Incubation Buffer

AMOUNT
1 ml Additional info:

PROTOCOL

. Protoplast Isolation - Incubation Buffer

CONTACT: Steven Burgess
Step 10.1.

Mannitol

AMOUNT
5 g Additional info:
Step 10.2.

0.5M MES, pH 5.6

AMOUNT
1 ml Additional info:

NOTES
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Final [10mM]

Step 10.3.

Potassium chloride

1M KCl

AMOUNT
200 µl Additional info:

NOTES
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Final [4mM]

Step 11.

Gently resuspend protoplast in incubation solution by light swirling of the test tube.

NOTES

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Note: You cannot view protoplasts on a flat glass slide with a coverslip as they will burst.

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Protoplasts are extremely fragile, it is best to avoid pipetting up and down or shaking vigorously as the shearing forces can cause cells to rupture.

Step 12.

Check protoplast integrity and determine yield using a disposable hemocytomer.

NOTES

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Diltute cells as necessary with extra incubation buffer.

Warnings

This protocol worked best for dicot species, yields from monocot leaves were low.