

Protoplast Isolation - Enzyme Buffer

Steven Burgess

Abstract

Enzyme solution for the isolation of protoplasts suitable for *Arabidopsis thaliana* and *Nicotiana benthamiana*. Adapted from Yoo et al.

2007 <http://www.nature.com/nprot/journal/v2/n7/full/nprot.2007.199.html>

And excellent video from the Sheen Lab is

available: <https://www.youtube.com/watch?v=5-xm1EoLrW4>

Citation: Steven Burgess Protoplast Isolation - Enzyme Buffer. **protocols.io**

[dx.doi.org/10.17504/protocols.io.fd2bi8e](https://doi.org/10.17504/protocols.io.fd2bi8e)

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Protocol

Step 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.

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 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 4.

Add dH₂O 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 20 μ M

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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 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 20 μ M

Step 6.

Macerozyme R10

[NOTES](#)

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Final Concentration is 0.3% (w/v)

Step 7.

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

Step 8.

1M Calcium Chloride

CaCl₂

☐ AMOUNT

50 µl Additional info:

Step 9.

Step 10.

Filter final solution through a 0.45-µm syringe filter.

Warnings

Solution must be made fresh.