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Product Information

Plant Protoplast Digest/Wash Solution

Catalog Number **D9692**

Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description

The Plant Protoplast Digest/Wash Solution is formulated to facilitate the rapid isolation of viable protoplasts from plant tissue. Plant cells are surrounded by a rigid, semi-permeable cell wall composed primarily of three classes of polysaccharides: cellulose, hemicellulose, and pectin.¹ The Plant Protoplast Digest/Wash Solution can be used for the digestion of the cell wall after the addition of enzymes that hydrolyze these polysaccharides, e.g., cellulase, pectinase, or pectolyase, and can subsequently be used to wash away any remaining hydrolytic enzymes after digestion is complete.

Plant protoplasts are typically used for any of a number of downstream applications. These applications include, but are not limited to, transient gene expression,² viral transfection assays,³ somatic hybridization,⁴ electrophysiological studies,⁵ and morphological studies.⁶

This simple protocol provides a high yield of protoplasts from commonly used plant species and tissues. The kit has been tested on a variety of plant species and tissue types.

Materials required, but not provided

Plant tissue
Hydrolytic enzymes
50 mL conical vials
Rotary shaker or rocker
Wide bore pipette tips
Centrifuge (swinging bucket)
Vortex mixer
Bright-Line Hemacytometer, Catalog Number Z359629
Microscope

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Stable for at least one year when stored at 2-8 °C.

Note: Numerous enzyme cocktails are cited in the literature for the isolation of protoplasts from a wide variety of plant species and tissue types. The type and concentration of hydrolytic enzymes that are used, as well as digestion times, may need to be optimized depending on the species and tissue(s) being studied. Many of these enzymes can be purchased in powdered form from Sigma. Some examples are listed below:

Catalog Number	Enzyme
C0615	Cellulase from <i>Trichoderma viride</i>
C8546	Cellulase from <i>Trichoderma reesei</i>
D8037	Driselase from <i>Basidiomycetes</i> sp.
P4300	Pectinase from <i>Rhizopus</i> sp.
P5936	Pectolyase from <i>Aspergillus japonicus</i>

These enzymes can be reconstituted directly in the Plant Protoplast Digest/Wash Solution as 1× to 10× cocktails. However, all enzyme cocktails should be passed through a 0.22 µm filter to remove any contaminating microorganisms. Increasing amounts of crude enzyme in the cocktail decreases filtering efficiency.

The protocol for the isolation of protoplasts from suspension cultures outlined below was optimized using *Arabidopsis* and *Nicotiana tabacum* cell suspension cultures. However, the Plant Protoplast Digest/Wash Solution has also been found to be suitable for the isolation of protoplasts from various tissues of a number of other plant species, including maize and canola.

Protoplast number and viability will vary between species and tissue types. Be aware that protoplasts are quite fragile in the absence of their cell walls – great care should be taken when handling them.

Procedure A

Protoplast isolation from suspension cultures

1. Collect an aliquot of the cell suspension in a 50 mL conical vial. The amount of cell suspension needed and the age of the culture will need to be determined experimentally. However, internal testing has shown that healthy protoplasts can be routinely obtained from 3-8 day-old cell suspension cultures.
2. Spin 10 min at 400 x *g* to pellet the cells, then remove the supernatant. Alternatively, you can allow the cells to settle for 15 to 20 min. The packed cell volume should be approximately 5 mL (to achieve this yield, culture conditions will need to be optimized as noted above).
3. Add Digest/Wash Solution to 50 mL, and mix by inversion.
4. Spin 10 min at 400 x *g* to pellet the cells (or allow cells to settle), then remove the supernatant.
5. Add Digest/Wash Solution containing your enzyme cocktail, and mix by inversion.
6. Gently agitate the mixture on a shaker or rocker at room temperature for 60 min. After the first 30 minutes, you may need to pipette gently several times with a wide bore pipette to break up clumps of tissue (a wide bore pipette should be used to avoid rupturing cells).
7. After 60 minutes, check for cell wall digestion and protoplast formation. Dilute a small aliquot of protoplasts 1:10 in Digest/Wash Solution (Add 50 μ L of the protoplast suspension to 450 μ L Digest/Wash solution). Place approximately 10 μ L of diluted suspension on a slide and cover with a glass coverslip. View the cells with a microscope. Digestion is complete when the majority of cells are spherical in shape.
8. Repeat step 7 every 30 minutes as necessary until at least 80% of the cells have formed protoplasts.
9. Spin 5 min at 100 x *g*. Remove the supernatant, making sure not to disturb the protoplast pellet.
10. Add Digest/Wash Solution to 50 mL, and mix by inversion.
11. Spin 5 min at 100 x *g*. Remove the supernatant, making sure not to disturb the protoplast pellet.
12. Determine the volume of the protoplast pellet, and add one volume of Digest/Wash Solution. Gently resuspend by inversion.
13. Incubate protoplasts on ice for at least 60 min prior to downstream treatments. This allows the cells time to recover from the digestion of their cell walls.

Procedure B

Protoplast isolation from leaf tissues

1. Store plants in the dark or in low light for 24 hrs before protoplast isolation. This will deplete starch reserves in the tissue (starch granules have been known to increase fragility of isolated protoplasts).
2. Collect 1 g of leaf tissue, and slice it into strips approximately 0.5-1 mm in width using a sharp scalpel or razor blade. Make sure not to bruise or tear the tissue, as this will lower the protoplast yield.
3. Place the leaf strips into a 50 mL conical vial, and add Digest/Wash Solution to 50 mL. Then, mix by inversion.
4. Remove Digest/Wash Solution with a pipette, making sure not to remove any leaf tissue.

Proceed with step 5 from Procedure A.

Procedure C

Protoplast counting

1. Dilute protoplasts 1:10 in Digest/Wash Solution by adding 50 μ L of the protoplast suspension to 450 μ L Digest/Wash solution. Gently invert the tube several times to ensure that the protoplasts are completely resuspended.
2. Count number of protoplasts using a Bright-Line hemacytometer. Gently mix the diluted protoplasts by inversion. Load each chamber of the hemacytometer and count the cells at 40 $\times$ magnification. Count only single, spherical cells as protoplasts.

Protoplast viability staining

The viability of the protoplasts can be determined by dual fluorescence staining with the Plant Cell Viability Assay Kit, Catalog Number PA0100.

Downstream procedures

Protoplasts are suitable for most downstream procedures. However, it is recommended that the protoplasts be washed and diluted to the appropriate concentration in a buffer solution recommended for a given downstream procedure.

References

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4. Ma, R., et al., Somatic embryogenesis and fertile green plant regeneration from suspension cell-derived protoplasts of rye (*Secale cereale* L.). *Plant Cell Rep.*, **22**, 320-7(2003).
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