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MasterPure™ Plant Leaf DNA Purification Kit

Cat. Nos. MPP92010 and MPP92100

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1. Introduction

The MasterPure™ Plant Leaf DNA Purification Kit[1] provides all of the reagents necessary to consistently isolate highly purified DNA from a variety of plant species. This kit combines a nonenzymatic approach to lyse the plant cell wall with a rapid precipitation step to remove macromolecules such as polyphenolics and polysaccharides. These contaminants in plant leaf nucleic acid preparations can interfere with restriction endonuclease digestion and PCR amplification of the DNA. DNA obtained with the MasterPure Plant Leaf DNA Purification Kit can be used in many applications including hybridization, restriction enzyme digestion, cloning, and PCR amplification. We offer several products for PCR that incorporate the MasterAmp™ PCR Enhancement Technology+, which substantially improves product yield and decreases nonspecific product formation.

2. Product Specifications

Storage: Store the MasterPure Plant Leaf DNA Purification Kit at room temperature.

Quality Control: The MasterPure Plant Leaf DNA Purification Kit is function-tested by extracting DNA from grapevine leaves. DNA quality and yield are assayed by agarose gel electrophoresis, fluorimetry, and use as a template for PCR.

The average total yield of DNA from 35 mg of fresh grapevine leaf is 500 ng.

3. Kit Contents

Desc.	Quantity
The MasterPure Plant Leaf DNA Purification Kit is available in 10- and 100-purification sizes. The 100-DNA purifications kit contains:	
Plant DNA Extraction Solution	35 ml
Cleanup Solution	25 ml
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)	6 ml

4. Related Products

The following products are also available:

- MasterPure™ Complete DNA and RNA Purification Kits
- MasterPure™ DNA Purification Kit
- MasterPure™ RNA Purification Kit
- MasterPure™ Yeast DNA Purification Kits
- MasterAmp™ Buccal Swab DNA Extraction Kits
- BuccalAmp™ DNA Extraction Kits
- MasterAmp™ PCR Optimization Kits
- MasterAmp™ *Taq*, *Tth*, *Tfl*, and AmpliTherm™ DNA Polymerases
- FailSafe™ PCR System

5. Plant Leaf DNA Purification Protocols

The following protocol is provided for the purification of DNA from plant leaves. Adjust reagent volumes proportionally for larger samples. Ribonuclease (available separately) may be used to remove RNA after completion of the protocol.

1. Grind 35-100 mg of fresh weight plant leaf in a 1.5-ml microcentrifuge tube containing 300 µl of Plant DNA Extraction Solution. We recommend using a micro-pestle designed for microcentrifuge tubes.
2. Incubate the ground tissue at 70°C for 30 minutes. Transfer samples onto ice and chill for 10 minutes.
3. Pellet cellular debris by centrifugation in a microcentrifuge for 5 minutes at ≥10,000 rpm.
4. Transfer the supernatant to a clean microcentrifuge tube; repeat the centrifugation step to remove residual debris. Transfer the supernatant to a clean microcentrifuge tube.
5. Add an equal volume of isopropanol to the clarified supernatant, and mix thoroughly by inversion. Pellet the DNA by centrifugation in a microcentrifuge for 5 minutes at ≥10,000 rpm.
6. Remove the supernatant with a pipet. Completely suspend the pelleted DNA in 100 µl of Cleanup Solution. Briefly vortex mix if necessary to ensure complete resuspension.
7. Add 100 µl of isopropanol to the resuspended DNA and mix thoroughly by inversion. Pellet the DNA by centrifugation in a microcentrifuge for 5 minutes at ≥10,000 rpm.
8. Wash the DNA pellet with 70% ethanol. Carefully remove and discard the ethanol with a pipet. Briefly centrifuge the DNA pellet and remove any remaining ethanol.
9. Suspend the DNA in 50 µl of TE buffer. If the DNA solution is not clear or colorless, repeat the cleanup steps by adding 100 µl of Cleanup Solution and precipitating with 150 µl of isopropanol (see Steps 7 and 8 above). Suspend the final pellet in 50 µl of TE buffer.
10. Quantitate DNA yield by fluorimetry using Hoechst dye 33258.[2] A_{260} estimates of yield can lead to gross overestimation of DNA content (up to 28 fold), even after ribonuclease treatment to degrade RNA.[1]
11. Store the DNA at 4°C.

6. References

1. Hoffman, L. and Moan, E. (1999) *Epicentre Forum* **6** (1), 1.
2. Ausubel, F. *et al.*, (eds.) (1995) *Current Protocols in Molecular Biology* (CD ROM ver. 3.7.5) John Wiley and Sons, New York, Appendix 3D.

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