

UltraClean™ Plant DNA Isolation Kit

Catalog # 13000-50
50 preps

Instruction Manual

Introduction

Use this kit for isolating DNA from 5-50mg plant samples.

Precautions

Please wear gloves when using this product. Avoid all skin contact with reagents in this kit. In case of contact wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or on our web site at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

This kit is for research purposes only. Not for diagnostic use.

Equipment required:

Micro centrifuge (10,000 x *g*)
Pipettor (volumes required 50 - 500µl),
Vortex

Kit Contents

<u>Description</u>	<u>Amt.</u>
2 ml Bead Solution tubes (contains 550µl solution)	50
Solution P1	3 ml
Solution P2	12.5 ml
Solution P3	45 ml
Solution P4	15 ml
Solution P5	2.5 ml
Spin filters units in 2.0 ml tubes	50
Collection tubes (2.0 ml)	150

Kit Storage

Room temperature

Make sure the 2ml Bead Solution screw cap tubes rotate freely in your centrifuge without rubbing.

WARNING: Solution P4 contains ethanol. It is flammable.

Protocol

Please wear gloves at all times

1. To the 2ml Bead Solution tubes provided, add 5-50mg of plant tissue. (Approximately one to ten samples of a hole punch).
 2. (Check Solution P1. If precipitated, heat to dissolve.)
 3. Add 60 μ l of Solution P1 and vortex once to mix. Upon adding, white precipitate may form initially, but will dissolve upon heating.
 4. Place the bead tubes in a water bath at 65°C for 10 minutes.
 5. Secure bead tubes horizontally using the MO BIO Tube Holder for the vortexer (cat. 13000-V1) or onto a flat-bed pad with tape.
 6. Vortex at maximum speed for 10 minutes.
 7. Make sure the 2ml tubes rotate freely in your centrifuge without rubbing.
 8. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.
 9. Transfer the supernatant to a clean microcentrifuge tube (provided).
Note: *With 50mg of plant tissue and depending upon plant type, expect 400 - 450 μ l of supernatant, which may contain some particles.*
 10. Add 250 μ l of Solution P2.
 11. Vortex 5 seconds.
 12. Incubate at 4°C for 5 min.
 13. Centrifuge sample tubes for 1 minute at 10,000 x *g*.
 14. Avoiding the pellet, transfer 500 μ l of supernatant to a clean microcentrifuge tube (provided).
 15. Add 1ml of Solution P3 to the supernatant.
 16. Vortex 5 seconds.
 17. **Note:** *A total of three loads for each sample processed are required* Load approximately 650 μ l onto spin filter and centrifuge at 10,000 x *g* for 30 seconds. Discard the flow through and add the remaining supernatant to the spin filter and centrifuge at 10,000 x *g* for 30 sec.
 18. Add 300 μ l of Solution P4.
 19. Centrifuge for 30 seconds at 10,000 x *g*.
 20. Discard the flow through.
 21. Centrifuge again for 1 minute to remove residual Solution P4.
 22. Carefully place spin filter in a new clean tube (provided). Avoid splashing any Solution P4 onto the spin filter.
 23. Add 50 μ l of Solution P5 to the center of the white filter membrane.
 24. Centrifuge 30 seconds.
 25. Discard spin filter. DNA in the tube is now application ready. No further steps are required.
- We recommend storing DNA frozen (-20°C). Solution P5 contains no EDTA.

Thank you for choosing the UltraClean Plant DNA Isolation Kit.

Version 03252005

Detailed Protocol (Explains what is happening at each step)

Please wear gloves at all times

1. Add 5-50mg of plant tissue to the 2ml Bead Solution Tube provided. (Approximately one to ten samples of a hole punch).

What's happening: Plant material is added to the Bead Solution Tube to prepare it for a bead beating homogenization step.

2. (Check Solution P1. If precipitated, heat to 65°C for several minutes to dissolve.)

What's happening: Solution P1 contains SDS. It will form a precipitate if it gets cold. Heating and dissolving the solids will restore it to full efficiency.

3. Add 60µl of Solution P1 and vortex once to mix. Upon adding, white precipitate may form initially, but will dissolve upon heating.

What's happening: If your plant samples are cold, Solution P1 may precipitate. This will not affect the DNA isolation procedure.

4. Place the bead tubes in a water bath at 65°C for 10 minutes.

What's happening: This heating step helps soften plant material prior to bead beating.

5. Secure bead tubes horizontally using the MO BIO Vortex Adapter Tube Holder for the vortexer (cat. 13000-V1) or onto a flat-bed pad with tape.

6. Vortex at maximum speed for 10 minutes. Be sure to check tubes periodically if you have taped them to a flat vortex pad. It is very common for tubes to shake loose. We recommend the use of the Mo Bio Vortex Adapter for this step.

What's happening: This bead beating step is a very good method to homogenize plant material without the need of grinding manually. In some cases the plant material will not be completely disintegrated after the 10 minute step. However, there should be sufficient disruption for a good to moderate yield of DNA.

7. Make sure the 2ml tubes rotate freely in your centrifuge without rubbing.

8. Centrifuge tubes at 10,000 x g for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.

What's happening: This step will pellet unwanted cell and tissue debris.

9. Transfer the supernatant to a clean microcentrifuge tube (provided).

What's happening: The supernatant contains DNA and other cell components. Avoid transferring any solid plant tissue at this point.

10. **Note:** With 50mg of plant tissue and depending upon plant type, expect 400 - 450µl of supernatant, which may contain some particles.

11. Add 250µl of Solution P2.

12. Vortex 5 seconds.

13. Incubate at 4°C for 5 minutes.

What's happening: Solution P2 is a protein precipitation reagent. It helps remove unwanted proteins

14. Centrifuge sample tubes for 1 minute at 10,000 x g.

What's happening: This step pellets the precipitated proteins.

15. Avoiding the pellet, transfer 500µl of supernatant to a clean microcentrifuge tube (provided).

16. Add 1ml of Solution P3 to the supernatant.

17. Vortex 5 seconds.

What's happening: Solution P3 is a binding salt. It is used to make the DNA bind to the silica spin filter membrane.

18. **Note:** A total of three loads for each sample processed are required. Load approximately 650µl onto spin filter and centrifuge at 10,000 x g for 30 seconds. Discard the flow through and add the remaining supernatant to the spin filter and centrifuge at 10,000 x g for 30 sec.

What's happening: In the presence of Solution P3 the binding salt, DNA will bind to the spin filter.

Spinning the SALT-DNA mixture through the spin filter leaves DNA bound to the filter membrane while allowing unwanted salt to pass through the membrane.

19. Add 300µl of Solution P4 to the spin filter.
20. Centrifuge for 30 seconds at 10,000 x g.
21. Discard the flow through.

What's happening: Solution P4 is a wash buffer. It removes residual salt and cleans the DNA. Solution P4 contains ethanol. It is flammable and should be discarded appropriately.

22. Centrifuge again for 1 minute to remove residual Solution P4.

What's happening: This is a critical step. It is very important to remove all traces of Solution P4 before continuing.

23. Carefully place spin filter in a new clean tube (provided). Avoid splashing any Solution P4 onto the spin filter.

24. Add 50µl of Solution P5 to the center of the white filter membrane.

25. Centrifuge 30 seconds.

What's happening: Solution P5 allows the bound DNA to be released from the spin filter membrane. It is a Tris buffer and contains no salt. DNA can not remain bound to the spin filter so it is released into the Solution P5 as it passes through the spin filter membrane.

26. Discard spin filter. DNA in the tube is now application ready. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution P5 contains no EDTA.

Thank you for choosing the UltraClean Plant DNA Isolation Kit.

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Hints and Troubleshooting Guide

Concentrating the DNA

Your final volume will be 50 μ l. If this is too dilute for your purposes, add 2 μ l of 5M NaCl or 5 μ l of 3M Sodium Acetate (pH: 5.2) and mix. Then add 100 μ l of 100% cold ethanol. Mix. Centrifuge at 10,000 x *g* for 5 minutes. Decant all liquid. Dry residual ethanol in a speed vac or dessicator or ambient air. Resuspend precipitated DNA in desired volume.

Amount of plant tissue to process

Depends on plant type. Usually 5-50 mg works well. (~1 to 6 hole punches of leaf tissue or equivalent).

DNA floats out of well when loaded on a gel

You may have inadvertently transferred some residual Solution P4 into the final sample. Prevent this by being careful in step 15 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation is the best way to remove residues of Solution P4. (See concentrating DNA above)

Storing DNA

DNA eluted in Solution P5 (10mM Tris) must be stored at -20°C or it may degrade. DNA can be eluted in TE but the EDTA may inhibit reactions such as PCR and automated sequencing.

**Other UltraClean™ Kits available from Mo Bio Laboratories, Inc.**

<u>Kit description</u>	<u>Cat. number</u>
Plasmid Prep Kits	
6 minute Mini Plasmid Prep Kit (100 preps)	12300-100
6 minute Mini Plasmid Prep Kit (250 preps)	12300-250
25-50 ml Plasmid Prep Kit (20 preps)	12700-20
25-50 ml Plasmid Prep Kit (50 preps)	12700-50
250-500 ml Plasmid Prep Kit (10 preps)	12600-10
250-500 ml Plasmid Prep Kit (20 preps)	12600-20
Endotoxin-Free Plasmid Prep Kits	
Endotoxin-free Mini Prep Kit (100 preps)	12311-100
Endotoxin-free Mini Prep Kit (250 preps)	12311-250
Endotoxin-free Midi Prep Kit (10 preps)	12711-10
Endotoxin-free Maxi Prep Kit (10 preps)	12611-10
DNA Purification Kits	
Agarose Gel DNA Purification Kit (300 preps)	12100-300
Agarose Gel-Spin DNA Purification (100 preps)	12400-100
Agarose Gel-Spin DNA Purification (250 preps)	12400-250
PCR Clean-Up Kit (100 preps)	12500-100
PCR Clean-Up Kit (250 preps)	12500-250
DNA Isolation Kits	
DNA Blood Isolation Kit (100 preps)	12000-100
DNA BloodSpin Kit (50 preps)	12200-50
DNA BloodSpin Kit (250 preps)	12200-250
Mega BloodSpin Kit (10 preps)	12210-10
Soil DNA Isolation Kit (50 preps)	12800-50
Soil DNA Isolation Kit (100 preps)	12800-100
Soil DNA Mega Prep Kit (10 preps)	12900-10
Fecal DNA Isolation Kit (50 preps)	12811-50
Fecal DNA Isolation Kit (100 preps)	12811-100
Microbial DNA Isolation Kit (50 preps)	12224-50
Microbial DNA Isolation Kit (250 preps)	12224-250
Plant DNA Isolation Kit (50 preps)	13000-50
Plant DNA Isolation Kit (250 preps)	13000-250
Tissue DNA Isolation Kit (50 preps)	12334-50
Tissue DNA Isolation Kit (250 preps)	12334-250
Water DNA Isolation Kit (10 preps)	14800-10
Water DNA Isolation Kit (25 preps)	14800-25
Forensic DNA Kit- Single prep format (10 preps)	14000-10
Forensic DNA Kit- Single prep format (20 preps)	14000-20
RNA Isolation Kits	
Tissue RNA Isolation Kit (50 preps)	15000-50
Tissue RNA Isolation Kit (250 preps)	15000-250
Plant RNA Isolation Kit (20 preps)	13300-20
Plant RNA Isolation Kit (50 preps)	13300-50
Microbial RNA Isolation Kit (50 preps)	15800-50
Microbial RNA Isolation Kit (250 preps)	15800-250
Growth Media	
TB DRY (1 kg) Terrific Broth powder	12105-1
LB (1 kg) LB powder (Miller)	12106-1
LB Agar (1 kg) LB Agar Powder (Miller)	12107-1



Technical information:

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Ordering Information

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For the distributor nearest you, go to our web site at www.mobio.com/distributors/