PowerPlant® Pro-htp
96 Well DNA Isolation Kit

Catalog No.	Quantity	Total Purifications
13496-4	4 Preps	384

Instruction Manual

Please recycle

Version: 03182013

Table of Contents

Introduction

The PowerPlant® Pro-htp 96 Well DNA Isolation Kit provides high-throughput isolation of high-quality DNA from 5-50 mg plant samples in less than 3 hours. The simple 96 well procedure provides highly reproducible yields of total cellular DNA. The streamlined procedure and convenient 96 well format eliminates tedious labeling, one-at-a-time pipetting and DNA resuspension steps, and helps minimize cross-contamination. Polysaccharides, proteins, and enzyme inhibitors are efficiently removed, making the purified DNA suitable for use in all downstream applications such as PCR, qPCR and next generation sequencing.

This kit requires the use of a specialized plate shaker in order to facilitate the bead beating process in the Metal Bead Plates. We recommend the Retsch 96 Well Plate Shaker (MO BIO Catalog# 11996 in the USA only) and Adapters (MO BIO Catalog# 11990). For information outside the USA, contact technical@mobio.com.

Phenolic Separation Solution (PSS)

For plant samples high in polyphenolic compounds, the addition of the Phenolic Separation Solution is recommended. The Phenolic Separation Solution prevents loss of nucleic acids by preventing irreversible binding to phenolics which are removed by IRT. Not all plant samples require the addition of PSS. Examples of plant tissues that require the addition of PSS are pine and grape leaf. If you are unsure of the phenolic content of your samples, evaluate the DNA recovery with and without PSS to determine the optimal protocol.

Average DNA Yields

DNA yields from plant tissues can vary based on the age and type of tissue and the level of phenolic compounds. Below are estimated DNA yields from a variety of plant samples evaluated using the PowerPlant® Pro-htp 96 Well DNA Isolation Kit.

Table 1.

Plant Sample	DNA yields (50mg)	PSS
Grape leaf	2.5-3.5 µg	+
Strawberry leaf	10-15 µg	+
Tomato stem	10-25 µg	+
Cotton leaf	2.5-3.5 µg	+/-
Cotton seed	20-25 µg	-
Grass leaf	40-50 µg	-
Pine needle	30-35 µg	+
Rice leaf	7-11 µg	-
Mint leaf	2-3 µg	-
Dried pepper leaf	10 µg	+/-
Soybean seed	5 µg	+
Watermelon, single seed	2.5 µg	+
Canola, 6 whole seeds	4 µg	+
Flax, 6 seeds with coat removed	2 µg	-
Wheat, single seed	10 µg	-
Barley, single seed	1.4 µg	-

+, yields were improved with the addition of PSS, **-,** yields were reduced with the addition of PSS, **+/-,** yields were the same with or without PSS.

Protocol Overview

Without the use of organic solvents like phenol and chloroform, the PowerPlant® Pro-htp 96 Well DNA Isolation Kit will isolate genomic DNA from plant tissues. Samples of plant tissue are added to a 96 well plate containing beads, bead solution, and lysis solution. The tissue is lysed by mechanical action on a 96 Well Plate Shaker. Patented Inhibitor Removal Technology® (IRT) is used to remove inhibitors from the lysates before binding the DNA to a spin plate containing silica membrane using centrifugation. The spin plate is washed to remove any contaminants and the DNA is finally eluted into 10 mM Tris buffer pH 8.

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

Other Related Products	Catalog No.	Quantity
96 Well Plate Shaker	11996	1 unit (120 V)
Plate Adapter Set	11990	1 set
Metal Bead Plates, 3.2 mm, 1 ml	13167-4	4 plates

Equipment Required

- Centrifuge capable of spinning two 96 well blocks stacked (13 cm x 8 cm x 5.5 cm) at 4500 x *g*
  Note: If you have a centrifuge with a maximum speed less than 4500 x *g* see the Hints and Troubleshooting Guide.
- Multi-channel Pipettor (volumes required 50 µl - 650 µl)
- Appropriate pipet tips for the Multi-channel pipettors.
  Note: The tips must fit in the round wells of the 1 ml blocks (examples of these are Molecular Bioproducts ART Catalog# 2179-HR, Eppendorf Catalog# 0030 077.750 and Rainin Catalog# RT-1000F).
- Mechanical Shaker that shakes 96 Well Blocks and Plate Adapter Set (MO BIO Catalog# 11996 and 11990)
- Vortex with 3 inch platform

Optional Equipment

Reagent reservoirs
Metal Bead Plates (MO BIO Catalog# 13167-4)

Kit Contents

	Kit Catalog# 13496-4	
Component	**Catalog #**	**Amount**
Solution PD1	13496-4-1	200 ml
Solution PD2	13496-4-2	22 ml
Solution PD3	13496-4-3	106 ml
Solution PD4	13496-4-4	255 ml
Solution PD5*	13496-4-5	106 ml
Solution PD7	13496-4-7	43 ml
RNase A Solution (25 mg/ml)	13496-4-8	1.5 ml
Phenolic Separation Solution	13496-4-9	17 ml
Spin Plates	13496-4-SP	4
2 ml Collection Plates	13496-4-2CP	4
1 ml Collection Plates	13496-4-1CP	4
0.5 ml Collection Plates	13496-4-0.5CP	8
Microplates	13496-4-MP	4
Centrifuge Tape	13496-4-CT	48
Sealing Tape	13496-4-ST	16
Elution Sealing Mats	13496-4-ESM	4

* **Add 106 ml of 100% ethanol to Solution PD5 before use!**

Kit Storage

RNase A should be stored at 4°C.
The other kit reagents and components should be stored at room temperature (15-30°C).

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 fire.

WARNING: Once Solution PD5, catalog #13496-4-5, is combined with the Ethanol it is flammable.

Experienced User Protocol

Please wear gloves at all times

This protocol assumes you will be processing 192 samples (2-96 well preps). If you plan to process less than this number, divide your samples between two plates evenly so that you always have a balance. See the Hints and Troubleshooting Guide.

- Add 106 ml of 100% ethanol to **Solution PD5** prior to starting to prepare the final wash solution. Check the box and write the date on the label.
- For steps 12 and 22 of this protocol, you will supply your own 100% ethanol. You need 106 ml of 100% ethanol per 96 well plate.
- Before starting, add RNase A Solution to Solution PD1 based on the number of samples you need to prep. Use **3 µl of RNase A Solution** per prep. Per 96 well plate, add **300 µl RNase A Solution to 45 ml of Solution PD1**.

1. If you are using the MO BIO Metal Bead Plates, remove the Round Well Mat and add up to 50 mg of wet plant material (or up to 10 mg of dry material) followed by the addition of **450 µl of Solution PD1/RNase A** if your sample is low in phenolics or **410 µl** of **Solution PD1/RNase A** and **40 µl of Phenolic Separation Solution (PSS)** if your sample is high in phenolics.

   See Table 1 on page 3 for guidelines on when to use PSS or call Technical Support (1-800-606-6246) if you have questions.

   Note: It is recommended that the tissue be cut into small pieces prior to loading it into the Metal Bead Plate. For tough plants or seeds pre-grind the material with a mortar and pestle**.**

   If you are using your own homogenization bead plate or method, follow the instructions above for addition of Solution PD1/RNase A to your wet or dry samples in your deep well block.

2. Check Solution PD2. If precipitated, heat to 60°C until dissolved before use. **Add 50 µl of Solution PD2**. Seal Bead Plate with Round Well Mat.

3. Place Bead Plate with mat securely fastened between 2 adapter plates (MO BIO Catalog# 11990) and place on the 96 Well Plate Shaker (MO BIO Catalog# 11996). Reference the protocol provided with the adapter plates for proper placement.

4. Shake at speed 20 for 8 minutes, remove plate and re-position so that the side closest to the machine body is now furthest from the machine body and shake again at speed 20 for 8 minutes.

   Note: This is a suggested starting point for many soft leaf tissue samples. Optimization of bead beating settings specific to your sample type is required.

5. Centrifuge 9 minutes at 4500 x *g*.

6. Remove and discard Round Well Mat. Transfer the supernatant to a clean 1 ml Collection Plate.

   Note: Expect between 450-550 µl of supernatant, depending on the plant type. The supernatant may still contain particles and debris.

7. Add **175 µl of Solution PD3** and place Sealing Tape onto plate. Vortex for 5 seconds.

   Note: For problematic samples you can add up to 250 µl of PD3 at this step. It is best to start at 175 µl for most sample types.

8. Incubate 1 ml Collection Plate at 4°C for 10 minutes.

9. Centrifuge the 1 ml Collection Plate for 9 minutes at 4500 x *g*. Remove and discard the Sealing Tape.

10. Avoiding the pellet, transfer the supernatant to a 2 ml Collection Plate.

11. Add **600 µl of Solution PD4** to the first row of the 2 ml Collection Plate containing the supernatant and pipet up and down to mix. Repeat for all remaining rows.

12. Add **600 µl of 100% ethanol** to the samples in the 2 ml Collection Plate. Pipette up and down to mix.

13. Seal any unused wells of the Spin Plate with Sealing Tape.

14. Place Spin Plate onto a new 0.5 ml Collection Plate.

15. Load approximately 600 µl onto the Spin Plate. Apply Centrifuge Tape.

16. Centrifuge for 5 minutes at 4500 x *g*.

17. Discard the flow through from the 0.5 ml Collection Plate and replace the same 0.5 ml Collection Plate beneath the Spin Plate. Discard the Centrifuge Tape.

18. Repeat steps 15 - 17 until all the supernatant has been processed (three loads).

19. Place the Spin Plate on a new 0.5 ml Collection Plate. **Add 500 µl of Solution PD5** to the Spin Plate.
    Note: Make sure 100% ethanol was added to the bottle of Solution PD5 prior to applying Solution PD5 to the Spin Plate.

20. Apply Centrifuge Tape. Centrifuge for 3 minutes at 4500 x *g*.

21. Discard the flow-through and replace the same 0.5 ml Collection Plate beneath the Spin Plate.

22. **Add 500 µl of 100% ethanol** to the Spin Plate. Apply a new piece of Centrifuge Tape.
    Note: In the PowerPlant® Pro DNA Isolation Kit (MO BIO Catalogt# 13400-50) 100% ethanol is provided and is called **Solution PD6**. Due to the large volumes of ethanol required for the 96 well kit, 100% ethanol is user provided.

23. Centrifuge for 3 minutes at 4500 x *g*.

24. Discard the flow-through and replace same 0.5 ml Collection Plate beneath the Spin Plate. Centrifuge for 7 minutes at 4500 x *g*.

25. Carefully place the Spin Plate on a Microplate. Remove the Centrifuge Tape and discard.

26. Allow to air dry for 10 minutes at room temperature.

27. **Add 100 µl of Solution PD7** (10 mM Tris pH 8) to the center of the white filter membrane of the Spin Plate. Apply a new piece of Centrifuge Tape. Allow Solution PD7 to sit on the membrane for 2 minutes at room temperature.

28. Centrifuge for 3 minutes at 4500 x *g*. Remove and discard the Centrifuge Tape. If all 96 wells were utilized you can discard the entire Spin Plate.

29. Seal the Microplate with the Elution Sealing Mat provided. DNA in the Microplate is now application ready. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution PD7 contains no EDTA.

Thank you for choosing the PowerPlant® Pro-htp 96 Well DNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)

Please wear gloves at all times

This protocol assumes you will be processing 192 samples (2-96 well preps). If you plan to process less than this number, divide your samples between two plates evenly so that you always have a balance. See the Hints and Troubleshooting Guide.

- Add 106 ml of 100% ethanol to **Solution PD5** prior to starting to prepare the final wash solution. Check the box and write the date on the label.
- For steps 12 and 22 of this protocol, you will supply your own 100% ethanol. You need 106 ml of 100% ethanol per 96 well plate.
- Before starting, add RNase A Solution to Solution PD1 based on the number of samples you need to prep. Use **3 µl of RNase A Solution** per prep. Per 96 well plate, add **300 µl RNase A Solution to 45 ml of Solution PD1.**

1. If you are using the MO BIO Metal Bead Plates, remove the Round Well Mat and add up to 50 mg of wet plant material (or up to 10 mg of dry material) followed by the addition of **450 µl of Solution PD1/RNase A** if your sample is low in phenolics or **410 µl** of **Solution PD1/RNase A** and **40 µl of Phenolic Separation Solution (PSS)** if your sample is high in phenolics.

   What's happening: Plant material is added to the Bead Plate to prepare it for a bead beating homogenization step. The PSS disassociates the phenolics from the nucleic acids so that they can be removed during the Inhibitor Removal Technology® (IRT) process.

   See Table 1 on page 3 for guidelines on when to use PSS or call Technical Support (1-800-606-6246) if you have questions.

   Note: It is recommended that the tissue be cut into small pieces prior to loading it into the Metal Bead Plate. For tough plants or seeds pre-grind the material with a mortar and pestle**.**

   If you are using your own homogenization bead plate or method, follow the instructions above for addition of Solution PD1/RNase A to your wet or dry samples in your deep well block.

2. Check Solution PD2. If precipitated, heat to 60°C until dissolved before use. **Add 50 µl of Solution PD2**. Seal Bead Plate with Round Well Mat.

   What's happening: Solution PD2 contains SDS. It will form a precipitate if it gets cold. Heating and dissolving the solids will restore it to full efficiency. Solution PD2 may be used while still warm.

3. Place Bead Plate with mat securely fastened between 2 adapter plates (MO BIO Catalog# 11990) and place on the 96 Well Plate Shaker (MO BIO Catalog# 11996). Reference the protocol provided with the adapter plates for proper placement.

4. Shake at speed 20 for 8 minutes, remove plate and re-position so that the side closest to the machine body is now furthest from the machine body and shake again at speed 20 for 8 minutes.

   Note: This is a suggested starting point for many soft leaf tissue samples. Optimization of bead beating settings specific to your sample type is required.

   What's happening: The bead beating step homogenizes plant material without the need for manual grinding. In some cases the plant material will not be completely disintegrated after the specified times of each method. However, there should be sufficient disruption for a good yield of DNA. The plate is rotated to ensure equal homogenization across all of the wells on the plate.

5. Centrifuge 9 minutes at 4500 x *g*.

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

6. Remove and discard Round Well Mat. Transfer the supernatant to a clean 1 ml Collection Plate.
   Note: Expect between 450-550 µl of supernatant, depending on the plant type. The supernatant may still contain particles and debris.

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

7. Add **175 µl of Solution PD3** and place Sealing Tape onto plate. Vortex for 5 seconds.
   Note: For problematic samples you can add up to 250 µl of PD3 at this step. It is best to start at 175 µl for most sample types.

8. Incubate 1 ml Collection Plate at 4°C for 10 minutes.

   What's happening: Solution PD3 is a novel formulation of Inhibitor Removal Technology® (IRT) and completes the process for removing PCR inhibitors in one step.

9. Centrifuge the 1 ml Collection Plate for 9 minutes at 4500 x *g*. Remove and discard the Sealing Tape.

   What's happening: This step pellets the proteins and inhibitors.

10. Avoiding the pellet, transfer the supernatant to a 2 ml Collection Plate.

11. Add **600 µl of Solution PD4** to the first row of the 2 ml Collection Plate containing the supernatant and pipet up and down to mix. Repeat for all remaining rows.

12. Add **600 µl of 100% ethanol** to the samples in the 2 ml Collection Plate. Pipette up and down to mix.

    What's happening: Solution PD4 is a binding salt. The concentration and amount of salt allows for optimal DNA binding to the silica spin filter membrane. 100% ethanol allows for maximal nucleic acid binding to the column.

13. Seal any unused wells of the Spin Plate with Sealing Tape.

14. Place Spin Plate onto a new 0.5 ml Collection Plate.

15. Load approximately 600 µl onto the Spin Plate. Apply Centrifuge Tape.

16. Centrifuge for 5 minutes at 4500 x *g*.

17. Discard the flow through from the 0.5 ml Collection Plate and replace the same 0.5 ml Collection Plate beneath the Spin Plate. Discard the Centrifuge Tape.

18. Repeat steps 15 - 17 until all the supernatant has been processed (three loads).

    What's happening: In the presence of Solution PD4 & 100% ethanol, DNA will bind to the spin filter plate. Centrifugation of the combined lysate through the spin filter allows the DNA to bind the filter membrane while allowing unwanted salt and impurities to pass through the membrane.

19. Place the Spin Plate on a new 0.5 ml Collection Plate. **Add 500 µl of Solution PD5** to the Spin Plate.
    Note: Make sure 100% ethanol was added to the bottle of Solution PD5 prior to applying Solution PD5 to the Spin Plate.

20. Apply Centrifuge Tape. Centrifuge for 3 minutes at 4500 x *g*.

    What's happening: Solution PD5 is an ethanol containing wash buffer that removes residual salt and other impurities from the spin filter membrane.

21. Discard the flow-through and replace the same 0.5 ml Collection Plate beneath the Spin Plate.

22. **Add 500 µl of 100% ethanol** to the Spin Plate. Apply a new piece of Centrifuge Tape.
    Note: In the PowerPlant® Pro DNA Isolation Kit (MO BIO Catalog# 13400-50) 100% ethanol is provided and is called **Solution PD6**. Due to the large volumes of ethanol required for the 96 well kit, 100% ethanol is user provided.

23. Centrifuge for 3 minutes at 4500 x *g*.

    What's happening: 100% ethanol is used to completely remove all residual salt from the spin filter membrane.

24. Discard the flow-through and replace same 0.5 ml Collection Plate beneath the Spin Plate. Centrifuge for 7 minutes at 4500 x *g*.

    What's happening: The ethanol remaining in the spin plate is dried using centrifugation. It is very important to remove all traces of the wash solutions before continuing.

25. Carefully place the Spin Plate on a Microplate. Remove the Centrifuge Tape and discard.

26. Allow to air dry for 10 minutes at room temperature.

    What's happening: This is a critical step. It is very important to allow all traces of the previous wash solutions to evaporate.

27. **Add 100 µl of Solution PD7** (10 mM Tris pH 8) to the center of the white filter membrane of the Spin Plate. Apply a new piece of Centrifuge Tape. Allow Solution PD7 to sit on the membrane for 2 minutes at room temperature.

    What's happening: Solution PD7 is 10 mM Tris, pH 8.0. The bound DNA is re-solubilized from the membrane into the low salt buffer that is neutral pH which protects DNA during storage. See the Hints and Troubleshooting Guide page 12 for more details.

28. Centrifuge for 3 minutes at 4500 x *g*. Remove and discard the Centrifuge Tape. If all 96 wells were utilized you can discard the entire Spin Plate.

29. Seal the Microplate with the Elution Sealing Mat provided. DNA in the Microplate is now application ready. No further steps are required.

    We recommend storing DNA frozen (-20°C). Solution PD7 contains no EDTA.

Thank you for choosing the PowerPlant® Pro-htp 96 Well DNA Isolation Kit.

Hints and Troubleshooting Guide

Processing less than 192 samples (less than 2 full plates)

This protocol assumes you will be processing 192 samples (2-96 well preps). If you plan to process less than this number, divide your samples between two plates evenly.

Distributing samples between two plates:

Balance the number of samples so centrifugation steps do not damage your centrifuge. It is best to match the total number of samples per plate as well as the orientation. For example, if you use wells A1-A12 in one plate, use those same wells in the second plate.

Multi-Channel pipettors:

The use of a multi-channel pipettor is advised for maximum efficiency. Most multi-channel pipettors are made to pipet multiples of 8 or 12 samples at a time. A multi-channel pipettor that can pipet a broad range of volumes is ideal. (Volumes required 50 µl - 650 µl).

Reagent reservoirs:

Use reagent reservoirs for the most efficient pipetting.

Mark used wells:

Be sure to mark all used wells to prevent reusing wells and cross contamination.

Centrifuge with a Maximum Speed Less Than 4500 x g

Multiply the protocol time and speed to determine total x *g*. Divide the total by the maximum speed of your centrifuge (round up if necessary). This will be the number of minutes your centrifuge will need to run to achieve the appropriate overall force.

Example: 10 minutes at 4500 x *g* = 45000.

If your centrifuge has a maximum speed of 2500 x *g*, divide 45000 ÷ 2500 = 18 minutes of centrifugation.

Concentrating the DNA

Your final volume will be 100 µl. If this is too dilute for your purposes, you can concentrate DNA by ethanol precipitation. This will be most efficient in smaller microcentrifuge tubes. Add 4 µl of 5M NaCl or 10 µl of 3M Sodium Acetate (pH: 5.2) and mix. Then add 200 µl of 100% cold ethanol. Incubate at -20°C for 10 minutes to overnight. Centrifuge at 13,000 x *g* for 15 minutes. Decant all liquid. Wash the DNA with 70% ethanol. Centrifuge for 10 minutes at 13,000 x *g*. Decant the ethanol and allow to dry in a speed vac or desiccator or ambient air. Resuspend precipitated DNA in desired volume.

Amount of plant tissue to process

This 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 100% ethanol from the last wash at step 22 into the final sample. Ethanol precipitation is the best way to remove residual ethanol (see “Concentrating the DNA” above)

Storing DNA

DNA eluted in Solution PD7 (10mM Tris) must be stored at -20°C or it may degrade. DNA can be eluted in TE-4 (10 mM Tris, 0.1 mM EDTA, Cat# 17320-1000) for long term storage and without any impact on enzymatic reactions.

Contact Information

Technical Support:
Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: technical@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

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

Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com

Description	Catalog No.	Quantity
PowerPlant® Pro DNA Isolation Kit	13400-50	50 preps
PowerPlant® RNA Isolation Kit	13500-50	50 preps
96 Well Plate Shaker	11996	1 unit (120 V)
Plate Adapter Set	11990	1 set
PowerMag™ Seed DNA Isolation Kit (Optimized for KingFisher®)	27700-4-KF	4 x 96 preps