Application Manual

Instruction Manual

FastDNA-96™ Plant & Seed DNA Kit

One Call

Rapid, High-Throughput Isolation of PCR - Ready Genomic DNA from Plant and Seed Samples using the FastPrep-96™ System

One Source

Catalog # 9696-600
2 x 96 Preps

A World of Biotechnology Reagents

Storage:
Ambient temperature (15 – 30°C)

Revision # 9696-600-11FEB

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1. Introduction to the FastDNA-96™ Plant & Seed DNA Kit and the FastPrep-96™ Instrument

The FastDNA-96™ Plant & Seed DNA Kit quickly and efficiently isolates inhibitor-free, PCR-ready genomic DNA from vegetative samples in approximately 50 minutes. Tough-to-lyse plant samples including stems, roots, leaves, buds, flowers, fruits and seeds are efficiently lysed in approximately 60 seconds with the FastPrep-96™ Instrument from MP Biomedicals.

The FastPrep-96™ Instrument is a high-throughput homogenizer developed to disrupt thoroughly any tissues and cells through the simultaneous bead-beating and impaction of specialized Lysing Matrix beads on the sample material. The FastPrep-96™ Instrument uses a linear vertical bidirectional motion providing an extremely quick and highly reproducible homogenization that surpasses early generation homogenizers, as well as traditional extraction methods using enzymatic digestion, sonication, blending, douncing or vortexing.

Samples are placed into 1.2 ml size tubes of a FastDNA-96™ Lysing Matrix Rack (96-deep well plate) containing 2.0 mm lysing matrix beads. The lysing matrix beads, which are specially stabilized Zirconium oxide particles, are designed to efficiently lyse a wide variety of Plantae kingdom specimens, while in the presence of a specially formulated lysis solution.

The FastDNA-96™ Plant & Seed DNA Kit isolates plant-source DNA from up to 80 mg samples, through a purification process that eliminates PCR inhibitors, polyphenols/tannins, and polysaccharides. Organic denaturants or proteinases are not needed with this procedure. Purified, inhibitor-free DNA is eluted in an EDTA-free, DNA elution solution, and is ready for

downstream applications including digestion, electrophoresis, arrays and PCR (A260/A280 ratios ≥ 1.8). Yield is typically 5 µg of total DNA eluted in 50-100 µl of elution solution.

The FastDNA-96™ Plant & Seed DNA Kit will recover genomic DNA fragments from 25 kb to 35 kb; however, some experiments have yielded results from as little as 100 bp up to >40 kb. Viral and parasitic DNA will also be isolated if these hosts are present in your samples.

2. Kit Components, Storage and User Supplied Materials

2.1 FastDNA-96™ Plant & Seed DNA Kit Components

FastDNA-96™ Lysing Matrix Rack (2.0 mm Beads)	2 x 96-well rack
Lysis Buffer	2 x 40 ml
Plant/Seed DNA Binding Solution	150 ml
Binding Plate Pre-Wash Buffer	50 ml
Plant/Seed DNA Wash Buffer	100 ml
Elution Solution	2 x 10 ml
Inhibitor Plate Prep Solution	30 ml
Deep-Well Plate	2 each
MP-96 Binding Plate	2 each
MP-96 Inhibitor Removal Plate	2 each
Collection Plate	2 each
Elution Plate	6 each
Foil Plate Cover	4 each
User manual	1 each
MSDS (Online: www.mpbio.com)	1 each
Certificate of Analysis	1 each

2.2 Storage

All FastDNA-96™ Plant & Seed DNA Kit reagents are stable at room temperature. Storage should be maintained at room temperature. The kit reagents are guaranteed for up to one year from the date of purchase of the kit.

2.3 User Supplied Materials

FastPrep-96™ Instrument (see Section 10)
Centrifuge with a swing-bucket style rotor that can spin up to 3,500 – 5,000 rpm
Swing-bucket centrifuge microplate adaptors for 96-well plates (pair)
Plate shaker
Sterile water

3. Important Considerations Before Use

3.1 Optimization of the Plant/Seed DNA Binding Solution

Prior to beginning the Kit Protocol in Section 5, the Plant/Seed DNA Binding Solution can be optimized for increased reagent performance and yield. Add 0.5% (v/v) beta-mercaptoethanol (750 µl to the 150 ml bottle) of the Plant/Seed DNA Binding Solution.

3.2 Binding Plate Pre-Wash Buffer

The FastDNA-96™ Plant & Seed DNA Kit contains a bottle of Binding Plate Pre-Wash Buffer which may form a precipitate over time. The precipitate is easily resuspended in solution by gently warming the bottle at 30 -37°C for up to 30 minutes with mixing by inversion. IMPORTANT: Do not microwave this reagent!

3.3 MP-96 Inhibitor Removal Plate Preparation

Prior to executing the protocol in Section 5, the MP-96 Inhibitor Removal Plate must be prepared for proper use in the assay. First mount the MP-96 Inhibitor Removal Plate on top of a supplied Elution Plate. Add 150 µl of Inhibitor Plate Prep Solution to the wells by puncturing the foil covering with the pipette tip. Incubate at room temperature for 5 minutes then centrifuge the stacked plates at 3,500 x g for 5 minutes. The MP-96 Inhibitor Removal Plate is now ready for sample preparation.

4. Safety Precautions

Some of the supplied kit reagents contain components that, when in contact with human tissue, may cause irritation. Wear personal protective equipment to prevent contact with the skin or mucus membranes (gloves, lab coat, and eye protection) at all times when using this product. Consult the Material Safety Data Sheet at www.mpbio.com for additional details. This product is for research purposes only.

5. Protocol

IMPORTANT NOTE BEFORE STARTING PROTOCOL:
When isolating DNA from plant or seed samples, the wells of the MP-96 Inhibitor Removal Plate must be prepared for proper use. Do not attempt this assay without first preparing the MP-96 Inhibitor Removal Plate or PCR-inhibitor contamination may occur. See Section 3.2, above, for instructions on preparing the MP-96 Inhibitor Removal Plate.

1. To the tubes of a FastDNA-96™ Lysing Matrix Rack, add up to 80 mg of finely cut plant or seed sample and 400 µl of Lysis Buffer per well. Re-cap the tubes.

2. Load the FastDNA-96® Lysing Matrix Rack into the FastPrep-96™ Instrument, and process the samples. A single 60 second run at a speed setting of 1600 rpm is sufficient to lyse almost all samples. If additional processing time is required over 5 minutes, the FastDNA-96™ Lysing Matrix Rack should be incubated on ice for at least 2 minutes between successive runs to prevent overheating the samples.

3. Place the FastDNA-96™ Lysing Matrix Rack in a microplate centrifuge adaptor and spin at 3,500 -5,000 x g for 5 minutes.

NOTE: Extending centrifugation up to 10 minutes can enhance elimination of excessive debris from fecal samples.

4. Transfer up to 250 µl of supernatant to the wells of a clean Deep-Well Plate.

5. Add 750 µl of Plant/Seed DNA Binding Solution to the

supernatant in each well of the Deep-Well Plate. Cover the wells of the Deep-Well Plate completely with the supplied Foil Plate Cover. Place the samples on a plate shaker or vortexer and shake/mix for 2 minutes.

6. Centrifuge the Deep-Well Plate for 5 minutes at 3,500 -5,000 x g.

7. Place the MP-96 Binding Plate on top of a supplied Collection Plate. Remove the foil from the Deep-Well Plate and transfer 500 µl of each supernatant to the wells of the MP-96 Binding Plate. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x g.

8. Discard the flow-through from the Collection Plate and re-use. Repeat Step 7 until all of the supernatant has been carefully transferred to the binding plate.

9. Continue to re-use the Collection Plate by placing it beneath the MP-96 Binding Plate. To the wells of the MP-96 Binding Plate, add 200 l of the Binding Plate Pre-Wash Buffer. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x g.

10. To the wells of the MP-96 Binding Plate, add 500 µl of Plant/ Seed DNA Wash Buffer. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x g.

11. Stack the MP-96 Binding Plate atop a clean Elution Plate. Add 50 - 100 µl of Elution Buffer directly to the matrix inside the wells of the MP-96 Binding Plate. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x g.

12. Stack a prepared MP-96 Inhibitor Removal Plate (see Section 3.2 for details) atop a clean Elution Plate. Transfer the eluent from Step 11 to the wells of the MP-96 Inhibitor Removal Plate. Centrifuge

the stacked plates for 5 minutes at 3,500 –5,000 x g.
13. Eluted DNA is now ready for PCR and other downstream applications. To store the samples, cover the Elution Plate with the supplied Foil Plate Cover. Store samples at 4°C until use, or at -20°C for extended periods.

6. Example Data: DNA Isolation from Plant & Seed Samples and Gel Electrophoresis

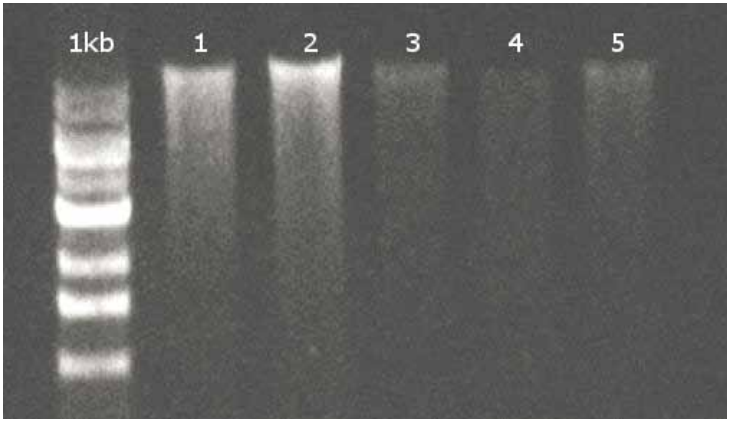

Figure 1a.
Figure 1a. samples include:
Lane 1: A. thaliana; 2: Juniper; Lane 3: Milkweed Leaf; Lane 4: Milkweed Leaflet; Lane 5: Milkweed Pre-Flowering Bud.

DNA purified from plant(a) and seed(b) using the FastDNA-96™ Plant & Seed DNA Kit. DNA was isolated from equivalent amounts of vegetative materials and loaded on a 0.8% agarose/ethidium bromide gel. (1 kb Ladder Marker, Zymo Research)

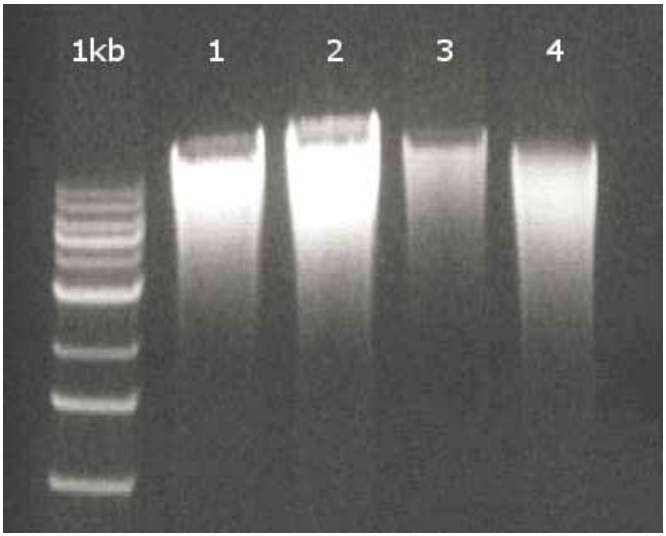

Figure 1b.
Figure 1b. samples include:
Lane 1-2: Corn Kernel; Lane 3-4: Sunflower Seed

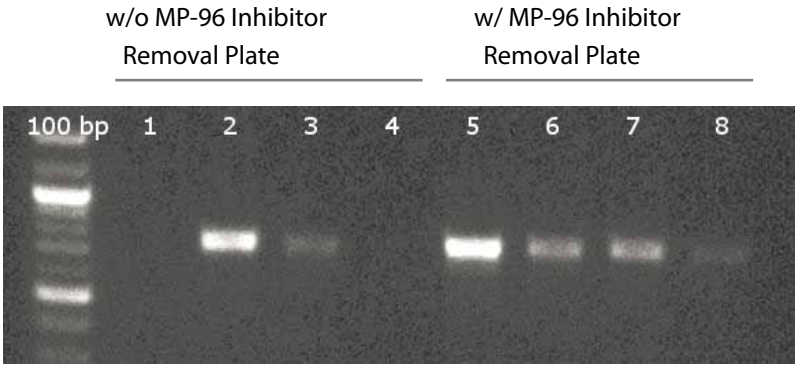

Figure 2.

PCR results of DNA extractions from Arabidopsis thaliana, illustrating the effectiveness of PCR-inhibitor removal by the MP-96 Inhibitor Removal Plate. The PCR was executed using DNA primers specific to a 700 bp amplicon of Chromosome 1. Lanes 1-4 and 5-8 exhibit decreasing volumetric dilutions of the PCR product (0 – 0.001, respectively). Amplicons were loaded on a 0.8% agarose/ethidium bromide gel. (100 bp Ladder Marker, Zymo Research)

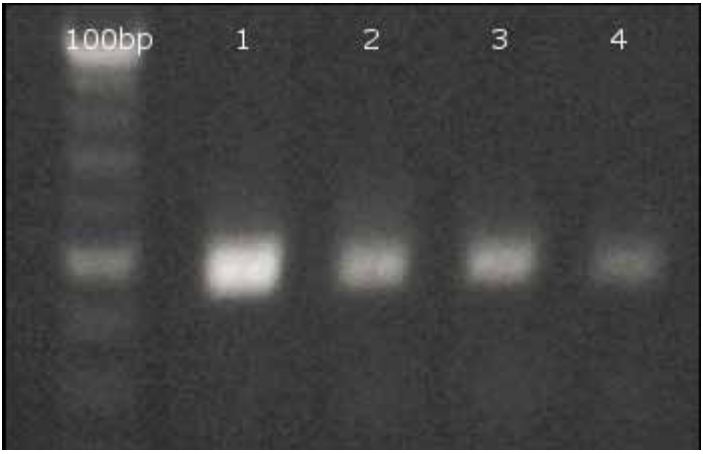

Figure 3.

PCR results of DNA extractions from corn kernels. The PCR was executed using DNA primers specific to a ~450 bp amplicon of mitochondrial DNA. Lanes 1-4 exhibit decreasing volumetric dilutions of the PCR product (0 – 0.001). Amplicons were loaded on a 0.8% agarose/ethidium bromide gel. (100 bp Ladder Marker, Zymo Research)

7. Recommended Reference Format for Publications

DNA was isolated from (specific sample) using the FastDNA-96™ Plant & Seed DNA Kit and the FastPrep-96™ Instrument (MP Biomedicals, Santa Ana, CA).

8. Technical Support

For technical support with this product please contact our MP Biomedical's Technical Support Team at 1-800-854-0530, by email at biotech@mpbio.com, or visit us online at www.mpbio.com for live support.

For our European customers, please contact our European Technical Support Team at 00 800 7777 9999, or by email at techsup.eur@mpbio.com.

9. Product Use Limitation & Warranty

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Revision # 9696-400-11FEB

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