

# Instruction Manual

## FastDNA-96™ Fungal & Bacterial DNA Kit

### One Call

High-Throughput, Rapid Isolation of PCR-Ready Genomic DNA from Tough-to-Lyse Fungi and Bacteria Samples using the FastPrep-96™ System

### One Source

Catalog # 9696-300  
2 x 96 Preps

### A World of Biotechnology Reagents

Storage:  
Ambient temperature (15 – 30°C)

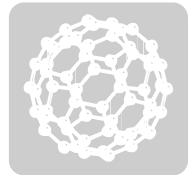
Revision # 9696-300-11FEB

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# FastDNA-96™ Fungal & Bacterial DNA Kit

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## 1. Introduction to the FastDNA-96™ Fungal & Bacterial DNA Kit and the FastPrep-96™ Instrument

The FastDNA-96™ Fungal & Bacterial DNA Kit quickly and efficiently isolates high-quality, PCR-ready genomic DNA from tough-to-lyse fungi samples and bacteria in approximately 40 minutes. The kit is designed for processing samples in 96-well plates and using the FastPrep-96™ Instrument from MP Biomedicals. Gram(+/-) bacteria, fungi, including *A. fumigatus*, *C. albicans*, *N. crassa*, *S. cerevisiae*, *S. pombe*, & mycelium, as well as spores, nematodes, pollen, and even mammalian cells are easily lysed in approximately 60 seconds.

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 0.5 mm lysing matrix beads. The lysing matrix beads, which are specially stabilized Zirconium oxide spheres, are designed to efficiently lyse all organisms including historically difficult sources such as eubacterial spores and endospores, gram (+/-) bacteria, fungi, yeast, molds, and algae, while in the presence of a specially formulated lysis solution.

The FastDNA-96™ Fungal & Bacterial DNA Kit isolates DNA from 10-20 mg of wet weight fungal or bacterial cells (approximately  $2 \times 10^8$  bacterial cells or  $2 \times 10^7$  yeast cells), or up to 40 mg soft animal tissue or Nematoda ( $2 \times 10^6$  mammalian cells). Organic denaturants

# FastDNA-96™ Fungal & Bacterial DNA Kit

or proteinases are not needed with this procedure. Purified DNA is eluted in an EDTA-free, DNA elution solution, and is ready for downstream applications including digestion, electrophoresis and PCR (A260/A280 ratios  $\geq 1.8$ ). Yield is typically 5  $\mu\text{g}$  of total DNA eluted in 50-100  $\mu\text{l}$  of elution solution (25 $\mu\text{l}$  minimum).

The FastDNA-96™ Fungal & Bacterial 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™ Fungal & Bacterial DNA Kit Components

FastDNA-96™ Lysing Matrix Rack (0.5 mm Beads) 2 x 96-well rack

Lysis Buffer	2 x 40 ml
Fungal/Bacterial DNA Binding Solution	150 ml
Binding Plate Pre-Wash Buffer	50 ml
Fungal/Bacterial DNA Wash Buffer	100 ml
Elution Solution	2 x 10 ml
Deep-Well Plate	2 each
MP-96 Binding Plate	2 each
Collection Plate	2 each
Elution Plate	2 each
Foil Plate Cover	4 each
User manual	1 each
MSDS (Online: <a href="http://www.mpbio.com">www.mpbio.com</a> )	1 each
Certificate of Analysis	1 each

## 2.2 Storage

All FastDNA-96™ Fungal & Bacterial 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

beta-mercaptoethanol

## 3. Important Considerations Before Use

### 3.1 Optimization of the Fungal/Bacterial DNA Binding Solution

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

### 3.2 Binding Plate Pre-Wash Buffer

The FastDNA-96™ Fungal & Bacterial DNA Kit contains a bottle of Binding Plate Pre-Wash Buffer which may form a precipitate over

# FastDNA-96™ Fungal & Bacterial DNA Kit

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!**

## 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](http://www.mpbio.com) for additional details. This product is for research purposes only.

## 5. Protocol

**NOTE:** Optimal reagent performance can be achieved by adding beta-mercaptoethanol to the Fungal/Bacterial DNA Binding Solution. See Section 3.2 for details of this optimization.

1. To the tubes of a FastDNA-96™ Lysing Matrix Rack, add 10-20 mg of wet weight bacterial or fungal cells, resuspended in up to 50 µl of sterile water, or sterile PBS. Add 400µl of Lysis Buffer per well and re-cap the tubes.

**NOTE:** 10-20 mg of wet weight cultured cells or tissue is equivalent to the following: 2x10<sup>8</sup> bacterial cells, 2x10<sup>7</sup> yeast cells, or 2x10<sup>6</sup> mammalian cells

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.

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

5. Add 750 µl of Fungal/Bacterial 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 an 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 200µl of the Binding Plate Pre-Wash Buffer. Centrifuge the stacked plates for 5 minutes at 3,500 - 5,000 x g.

# FastDNA-96™ Fungal & Bacterial DNA Kit

10. To the wells of the MP-96 Binding Plate, add 500 µl of Fungal/Bacterial 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 (25µl minimum) 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. 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 Bacteria & Yeast Samples and Gel Electrophoresis

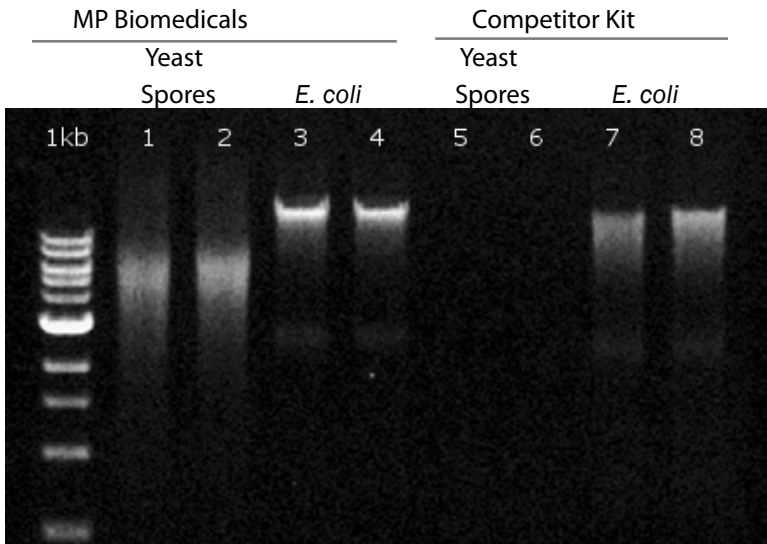


Figure 1.

Comparison of DNA from *S. cerevisiae* and *E. coli* cells extracted with the FastDNA-96™ Fungal & Bacterial DNA Kit and a competitor kit. Samples were loaded on a 0.8% agarose/ethidium bromide gel (1 kb Ladder Marker, NEB).

## 7. Recommended Reference Format for Publications

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

## 8. Technical Support

For technical support with this product please contact our MP Biomedicals's Technical Support Team at 1-800-854-0530, by email at [biotech@mpbio.com](mailto:biotech@mpbio.com), or visit us online at [www.mpbio.com](http://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](mailto:techsup.eur@mpbio.com).

## 9. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and the sole liability of MP Biomedicals hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification. By acceptance of the product, Buyer indemnifies and holds MP Biomedicals harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s). GeneClean® and FastPrep® are registered trademarks of MP Biomedicals, LLC.



## Other FastPrep® Kits Available

FastDNA® Kit and FastDNA® Spin Kit

Cat N° 6540-400 - Cat N° 6540-600 respectively (100 preps)

- Lyse and isolate DNA in less than 30 minutes
- Plant, animal, yeast, fungal and microbial samples
- No hazardous organic reagents required
- SPIN filters streamline silica handling (FastDNA Spin Kit)

FastDNA® Spin Kit for Feces

Cat N° 6570-200 (50 preps)

- Lyse and isolate PCR-ready DNA in less than 30 minutes
- Variety of stool sample types
- No hazardous organic reagents required
- SPIN filters streamline silica handling

FastDNA® 50ml Spin Kit for Soil

Cat N° 6560-600 (10 preps)

- Process low-microbial containing soil samples.
- Lyse and isolate PCR-ready DNA in less than 60 minutes
- Variety of soil and environmental sample types
- No hazardous organic reagents required
- SPIN filters streamline silica handling

FastRNA® Pro Blue Kit

Cat N° 6025-050 (50 preps)

- For use with gram positive and gram negative bacteria
- Lyse up to 10<sup>10</sup> cells per 2ml tube
- Lysis and isolation with single-phase organic solution in less than 90 minutes

### FastRNA® Pro Red Kit

Cat N° 6035-050 (50 preps)

- For use with yeast cells and fungal tissue
- Lyse up to 1010 cells per 2ml tube
- Lysis and isolation with single-phase organic solution in less than 90 minutes

### FastRNA® Pro Green Kit

Cat N° 6045-050 (50 preps)

- For use with all plant and animal samples
- Lyse 50-100 mg tissue per 2ml tube
- Lysis and isolation with single-phase organic solution in less than 90 minutes

### FastRNA® Pro Soil-Direct Kit and FastRNA® Pro Soil-Indirect Kit

Cat N° 6070-050 - Cat N° 6075-050 respectively (50 preps)

- Isolate RNA from soil samples (direct kit) and washed soil (indirect kit) in less than 2 hours
- Variety of soil and environmental sample types
- RNA protected during and after processing
- Humic acids reduced to allow uninhibited RT-PCR
- Includes additional reagents for even further purification if necessary
- SPIN filters streamline silica handling

### FastProtein™ Blue Matrix

Cat N° 6550-400 (50 preps) - Cat N° 6550-500 (100 preps)

- Release of proteins from gram positive and gram negative bacteria in 40 seconds
- Protein extracts are ready for immediate electrophoresis or purification
- Ideal for optimizing induction conditions

### FastProtein™ Red Matrix

Cat N° 6550-600 (50 preps) - Cat N° 6550-700 (100 preps)

- Release of proteins from yeast cells and fungi in 40 seconds
- Protein extracts are ready for immediate electrophoresis or purification
- Ideal for optimizing induction conditions

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