One Call

Instruction Manual FastDNA-96[™] Tissue & Insect DNA Kit

High-Throughput, Rapid Isolation of PCR-Ready Genomic DNA from Animal and Insect Samples using the FastPrep-96[™] System

> Catalog # 9696-500 2 x 96 Preps

Storage: Ambient temperature (15 – 30°C)

Revision # 9696-500-11FEB

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TABLE OF CONTENTS

1. Introduction to the FastDNA-96™Tissue & Insect DNA Kit	
and the FastPrep-96™ Instrument	5
2. Kit Components, Storage, & User Supplied Materials	6
2.1 FastDNA-96™ Tissue & Insect DNA Kit Components	6
2.2 Storage	7
2.3 User Supplied Materials	7
3. Important Considerations Before Use	7
3.1 Optimization of the Tissue/Insect DNA Binding Solution .	7
3.2 Binding Plate Pre-Wash Buffer	8
4. Safety precautions	8
5. Protocol	8
6. Example Data: DNA Isolation from Tissue & Insect Samples	
and Gel Electrophoresis	10
7. Recommended Reference Format for Publication	11
8. Technical Support	11
9. Product Use Limitation & Warranty	12



1. Introduction to the FastDNA-96[®] Animal & Insect DNA Kit and the FastPrep-96[®] Instrument

The FastDNA-96[™] Tissue & Insect DNA Kit quickly and efficiently isolates highly pure, PCR-ready genomic, viral and mitochondrial DNA from fresh or frozen tissue samples, insects, and arthropods in approximately 40 minutes. The kit is designed for processing samples in 96-well plates and using the FastPrep-96[™] Instrument from MP Biomedicals. Animal tissues (brain, heart, kidney, liver, bone, etc), cultured mammalian cells, whole blood, as well as D. melanogaster, mosquitoes, bees, lice, and ticks 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 2.0 mm lysing matrix beads. The lysing matrix beads, which are specially stabilized Zirconium oxide spheres, are designed to efficiently lyse all cell and tissue types whether mammalian or invertebrate, while in the presence of a specially formulated lysis solution.

The FastDNA-96[™] Tissue & Insect DNA Kit isolates DNA from 1 -10 mg of fresh, frozen, or stored mammalian tissue or insects. Organic denaturants 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, arrays, genotyping, and PCR (A260/A280 ratios \geq 1.8). Yield is typically 1-5 µg of DNA per mg tissue (3-7µg of DNA per 100 µl whole blood) and is eluted in 50-100 µl of elution solution (25µl minimum).

The FastDNA-96[™] Tissue & Insect 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

FastDNA-96 [™] Lysing Matrix Rack (2.0 mm Beads) 2 x 96-well rack		
Lysis Buffer	2 x 40 ml	
Tissue/Insect DNA Binding Solution	150 ml	
Binding Plate Pre-Wash Buffer	50 ml	
Tissue/Insect 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: www.mpbio.com)	1 each	
Certificate of Analysis	1 each	



2.2 Storage

All FastDNA-96 Tissue & Insect 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 Sterile PBS beta-mercaptoethanol

3. Important Considerations Before Use

3.1 Optimization of the Tissue/Insect DNA Binding Solution

Prior to beginning the Kit Protocol in Section 5, the Tissue/Insect DNA Binding Solution can be optimized for increased reagent performance and yield. Add 0.5% (v/v)

B-mercaptoethanol (750µl to the 150 ml bottle) to the Tissue/ Insect DNA Binding Solution.

3.2 Binding Plate Pre-Wash Buffer

The FastDNA-96[™] Tissue/Insect 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!

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

NOTE: Optimal reagent performance can be achieved by adding B-mercaptoethanol to the Tissue/Insect DNA Binding Solution. See Section 3.2 for details of this optimization.

1. To the tubes of a FastDNA-96[™] Lysing Matrix Rack, add 1-10 mg of fresh or frozen mammalian or insect tissue, 1.7x106 mammalian cultured cells resuspended in 50-100 µl sterile PBS, or up to 100 µl whole blood. Add 400 µl of Lysis Buffer per well and re-cap the tubes.

NOTE: Not more than 10 mg of tissue should be sampled. The



DNA binding capacity of the MP-96 Binding Plate will be exceeded in samples above 10 mg starting tissue.

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^m Lysing Matrix Rack in a microplate centrifuge adaptor and spin at 3,500 -5,000 x g for 5 minutes.

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

5. Add 750 μ l of Tissue/Insect 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 μ l 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 reuse. Repeat Step 7 until all of the supernatant has been carefully transferred to the binding plate.

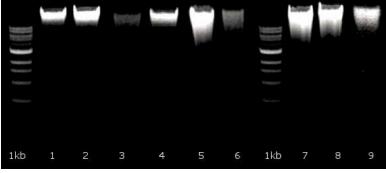
9. Continue to re-use the Collection Plate by placing it beneath the MP-96 Binding Plate. To the wells of the MP-96 Binding Plate, add 200 μ l of the Binding Plate Pre-Wash Buffer. Centrifuge the stacked plates for 5 minutes at 3,500 - 5,000 x g.

10. To the wells of the MP-96 Binding Plate, add 500 μ l of Tissue/ Insect 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 μ l of Elution Buffer (25 μ l 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 Tissue & Insect Samples and Gel Electrophoresis







Comparison of DNA from insects (left) and mammalian tissues (right) extracted with the FastDNA-96[™] Tissue & Insect DNA Kit. Samples were loaded on a 0.8% agarose/ethidium bromide gel (1 kb Ladder Marker, Zymo Research).

Figure 1. samples include:

Lane 1: D. melanogaster(n=10); 2: D. melanogaster(n=20); Lane 3: D. melanogaster larvae(n=10); Lane 4: D. melanogaster larvae(n=20); Lane 5: Darkling Beetle larva; Lane 6: Cricket; Lane 7: Mouse Kidney; Lane 8: Mouse Liver; Lane 9: Mouse Tail.

7. Recommended Reference Format for Publications

DNA was isolated from (specific sample) using the FastDNA-96[™] Tissue & Insect 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

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

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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 1010 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

Application Manual

Revision # 9696-400-11FEB

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