Instruction Manual

FastDNA® Green SPIN Kit

Rapid Isolation of Genomic DNA from Plant and Animal Tissues Using the FastPrep® System

› *One Call*

› *One Source*

› *A World of Biotechnology Reagents*

100 Preps

Storage:
Ambient temperature (15 – 30°C)

Catalog # 6540-800

Revision # 6540-800-08FEB

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24 x 2 ml samples

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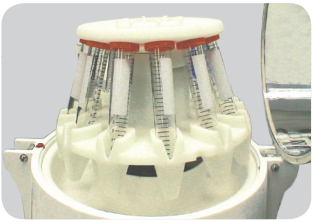

HighPrep™ Adapter
48 x 2ml samples

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1. Introduction to the FastDNA® Green SPIN Kit and the FastPrep® Instruments

The FastDNA® Green SPIN Kit quickly and efficiently isolates genomic DNA from a wide variety of animal and plant tissue sources. Designed for use with the FastPrep® Instruments from MP Biomedicals, plant and animal tissues, are easily lysed within 40 seconds. These benchtop devices use a unique, optimized motion to homogenize samples by multidirectional, simultaneous impaction with lysing matrix particles. FastPrep® Instruments provide an extremely quick, efficient and highly reproducible homogenization that surpasses traditional extraction methods using enzymatic digestion, sonication, blending, douncing and vortexing.

Samples are placed into 2.0 ml tubes containing the proprietary Lysing Matrix D Matrix, irregularly shaped. While almost all samples are easily processed with this pre-filled combination, additional ¼ inch ceramic spheres are provided for hard samples such as bone, cartilage or seeds.

Homogenization in the FastPrep® Instrument with Lysing Matrix D takes place in the presence of sample-specific Cell Lysis Solutions (CLS). For plant tissues, CLS-VF is used in conjunction with a Protein Precipitation Solution (PPS). For all other samples including human and animal tissues, CLS-TC is used during sample lysis. For maximum flexibility, all buffers are provided in the kit.

Following lysis, samples are centrifuged to pellet debris and lysing matrix. DNA is purified from the supernatant with a silica-based GENECLEAN® procedure using SPIN filters. Eluted DNA is ready for digestion, electrophoresis, PCR and any other desired application.

2. Kit Components and User Supplied Materials

2.1 FastDNA® Green SPIN Kit Components

Lysing Matrix D	100x 2.0 ml tubes
¼ Ceramic Spheres	100 spheres
Binding Matrix	66 ml
Concentrated SEWS-M	12 ml
DES	25 ml
CLS-VF	90 ml
PPS	25 ml
CLS-TC	110 ml
CLS-Y	110 ml
SPIN Modules	50 each
Catch Tubes	50 each
User manual	1 each
MSDS	1 each
Certificate of Analysis	1 each

2.2 User Supplied Materials

FastPrep® Instrument (see Section 10)
Microcentrifuge that can freely spin 2.0 ml tubes
Microcentrifuge tubes (2.0 ml and 1.5 ml)
Rotator or low-speed vortex

3. Important Considerations Before Use

3.1 Preparation of SEWS-M Wash Solution

The FastDNA® Green SPIN Kit contains a bottle with 12 ml of Concentrated SEWS-M Wash Solution. Before using this solution, add 100 ml of 100% ethanol and mark on the bottle label the date ethanol was added. Ensure that the bottle is securely closed to prevent evaporation, and store at room temperature.

3.2 Precipitation in CLS-TC Buffer

If the FastDNA® Green SPIN Kit was shipped or stored at a low temperature, a harmless precipitate may form in the CLS-TC Buffer. If a precipitate is seen, incubate the bottle in a 45-55°C water bath for several minutes and mix to bring the precipitate back into solution. Allow solution to cool to room temperature.

3.3 Sample Lysis with the FastPrep® Instrument

The fill volume in the lysing matrix tube after the addition of the Cell Lysis Solution to the sample should allow sufficient air space in the sample tube for efficient FastPrep® Instrument processing. MP Biomedicals recommends using 100 – 200 mg of starting material as long as there is between 250 – 500 µl of empty space in the tube. Sample loss or tube failure may result from overfilling the matrix tube. The matrix tube caps must be secure, but not over-tightened, to prevent sample leakage. If the sample is too large for processing in a single tube, divide the sample and process using multiple tubes.

MP Biomedicals' Lysing Matrix particles and tubes have been rigorously tested and validated in the FastPrep® Instrument. The use of other products with the FastPrep® Instrument is not recommended and may result in sample loss or instrument failure.
A single 40 second run at a speed setting of 6.0 in the FastPrep® Instrument is sufficient to lyse almost all samples. If the user experimentally determines that additional processing time is required, the sample should be incubated on ice in the Lysing Matrix D tube for at least 2 minutes between successive FastPrep® Instrument homogenizations to prevent overheating the sample and tube.

MP Biomedicals recommends that all researchers begin the protocol with the Lysing Matrix D as supplied in the kit (garnet matrix and single sphere). If lysis is inefficient even after multiple runs of 40 seconds, an additional ¼ inch ceramic sphere (provided) can be added on top of the sample. Depending on the sample, lysis and/or yield may or may not improve and shearing of existing genomic DNA may begin to occur. Samples with an additional 1/4 inch ceramic sphere should be processed carefully in order to balance increased yield and lysis against increased DNA shearing by varying speed and/or time settings.

3.4 Recovery of DNA from Dry Samples

To optimize DNA recovery from extremely dry samples, leave the lysed sample at room-temperature in the Lysing Matrix D tube for an incubation period of 15 minutes to 2 hours after processing in the FastPrep® Instrument.

3.5 Co-Purification of RNA

Some tissues (i.e. liver, kidney) contain very high levels of RNA which may co-purify with the genomic DNA. If absolute control of RNA contamination is necessary, the final eluted DNA can be treated with RNase as per the manufacturer's protocol.

4. Safety Precautions

Binding Matrix contains 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). Consult the enclosed Material Safety Data Sheet for additional details.

5. Protocol

1. Add sample to Lysing Matrix D tube. Place up to 100 - 200 mg tissue (fresh, frozen, dried etc.), or 200 µl of cells suspended in water or isotonic saline solution. For blood, lymph or tissue culture cells grown in suspension : Centrifuge a sufficient volume of culture to provide a pellet size of 50-100 mg wet weight or up to 10^7 mammalian or plant cells. Resuspend pellets in water or isotonic saline to give a maximum suspension volume of 200 µl.

   NOTE: See section 3.3 for other important guidelines.

2. Add appropriate Cell Lysis Solution (CLS) according to table below:
   Plant tissues 800 µl CLS-VF and 200 µl PPS
   Human and animal tissues, cultured cells, 1.0 ml CLS-TC

3. Homogenize in the FastPrep® Instrument for 40 seconds at a speed setting of 6.0.

4. Centrifuge at 14,000 x g for 5-10 minutes to pellet debris.

5. Transfer supernatant (700 – 800 µl) to a 2.0 ml microcentrifuge tube and add an equal volume of Binding Matrix. Invert to mix.

   NOTE: It is important to use a tube that is large enough to allow room for complete mixing of the entire volume during the course of the next step. Tubes with conical bottoms are not recommended. A 2.0 ml microcentrifuge tube works well at this step.

6. Incubate with gentle agitation for 5 minutes at room temperature on a rotator.

   NOTE: A low-speed vortex may be used at this point, but care must be taken not to shear the DNA.

7. Transfer half (approximately 800 µl) of the suspension to a SPIN™ Filter and centrifuge at 14,000 x g for 1 minute. Empty the catch tube and add the remaining suspension to the SPIN™ Filter and centrifuge as before. Empty the catch tube again.

8. Add 500 µl prepared SEWS-M and gently resuspend the pellet using the force of the liquid from the pipet tip.

   NOTE: Ensure that ethanol has been added to the Concentrated SEWS-M. See section 3.1.

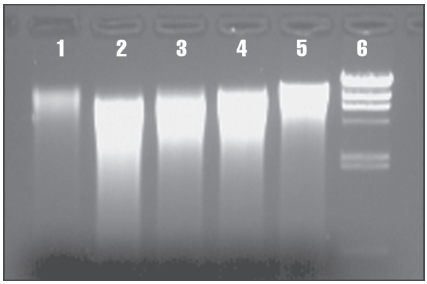
9. Centrifuge at 14,000 x g for 1 minute. Discard contents of Catch Tube and replace.

10. Without any addition of liquid, centrifuge a second time at 14,000 x g for 2 minute and replace the Catch Tube with a new, clean tube.

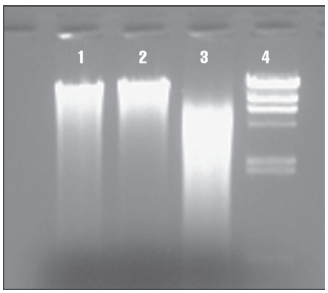
11. Elute DNA by gently resuspending Binding Matrix above the SPIN filter in 100 µl of DES. Incubate for 5 minutes at 55˚C in a heat block or water bath.

12. Centrifuge at 14,000 x g for 1 minute to bring eluted DNA into the clean catch tube. Discard the SPIN filter. DNA is now ready for downstream applications. Store at -20°C for extended periods or 4°C until use.

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



DNA from plant samples extracted with the FastDNA® Kit. Approximately 1 µg of isolated DNA was loaded on a 1.2% agarose gel (0.5X TAE). Lane 1: ≈0.16g apple stem; Lane 2: ≈0.45g red bell pepper seeds; Lane 3: ≈0.45g pelargonium root; Lane 4: ≈0.45g mature peace lily leaf; Lane 5: ≈0.45g ice plant leaf; Lane 6: Lambda Hind III marker.



DNA from animal samples extracted with the FastDNA® Kit. Approximately 1 µg of isolated DNA was loaded on a 1.2% agarose gel (0.5X TAE). Lane 1: ≈0.4g rat liver; Lane 2: ≈0.5g mouse brain; Lane 3: ≈0.45g chicken bone; Lane 4: Lambda Hind III marker.

7. Table of Typical FastPrep® Settings

Sample Name	Sample Type	Quantity	Lysing Matrix	FastPrep® speed	FastPrep® time
HUMAN AND ANIMAL					
Human	Lung	50 mg	Lysing Matrix D	6.0	4x 30 sec.
Human	Breast	80 mg	Lysing Matrix D	6.0	2x 30 sec.
Human	Kidney	50 mg	Lysing Matrix D	6.0	40 sec.
Human	Thyroid Tumors	100 mg	Lysing Matrix A	6.0	3x 30 sec.
Mouse	Eye	10 mg	Lysing Matrix D	6.0	4x 30 sec.
Mouse	Heart	70 mg	Lysing Matrix D	6.0	4x 30 sec.
Mouse	Kidney	50 mg	Lysing Matrix D	6.0	40 sec.
Mouse	Femur	40 mg	Lysing Matrix A	6.0	4 x 30 sec.
Mouse	Leg Muscle	50 mg	Lysing Matrix D	6.0	40 sec.
Mouse	Intestine	50 mg	Lysing Matrix D	6.0	40 sec.
Mouse	Ear	45 mg	Lysing Matrix D	6.0	4x 30 sec.
Mouse	Tail	100 mg	Lysing Matrix A	6.0	4x 30 sec.
Mouse	Spleen	70 mg	Lysing Matrix D	6.0	40 sec.
Mouse	Lung	50 mg	Lysing Matrix D	6.0	40 sec.
Mouse	Liver	50 mg	Lysing Matrix D	6.0	40 sec.
Mouse	Brain	50 mg	Lysing Matrix D	6.0	40 sec.
Mouse	Pancreatic cells (bHC9)	10^7 cells	Lysing Matrix D	6.0	40 sec.
PLANT					
Alpowa Wheat	Leaf Tissue	75 mg	Lysing Matrix D	6.0	40 sec.
Alpowa Wheat	Seed	100 mg	Lysing Matrix A	6.0	40 sec.
Arabidopsis thaliana	Fresh Leaves	50 mg	Lysing Matrix D	6.0	40 sec.
Arabidopsis thaliana	Fresh Leaves	200 mg	Lysing Matrix D	6.0	2x 40 sec.
Bartlett Pear	Leaf Tissue	50 mg	Lysing Matrix D	6.0	40 sec.
Classic Oat	Leaf Tissue	75 mg	Lysing Matrix D	6.0	40 sec.
Classic Oat	Seed	100 mg	Lysing Matrix A	6.0	40 sec.
Corn	Leaf Tissue	100 mg	Lysing Matrix D	6.0	40 sec.
Crest Barley	Leaf Tissue	100 mg	Lysing Matrix D	6.0	40 sec.
Crest Barley	Root	300 mg	Lysing Matrix A	6.0	40 sec.
Kaybonnet Rice	Leaf Tissue	100 mg	Lysing Matrix D	6.0	40 sec.

Sample Name	Sample Type	Quantity	Lysing Matrix	FastPrep® speed	FastPrep® time
Kaybonnet Rice	Seed	100 mg	Lysing Matrix A	6.0	40 sec.
Klages Barley	Root	300 mg	Lysing Matrix A	6.0	40 sec.
Klages Barley 70 mg Leaf Tissue 6.0 40 seconds	Leaf Tissue	70 mg	Lysing Matrix D	6.0	40 sec.
Tobacco	Leaf Tissue	75 mg	Lysing Matrix D	6.0	40 sec.
Lafitte Rice	Leaf Tissue	75 mg	Lysing Matrix D	6.0	40 sec.
Lafitte Rice	Sprout Leaf	100 mg	Lysing Matrix D	6.0	2x 30 sec.
Soybean	Seed	100 mg	Lysing Matrix A	6.0	40 sec.
Corn	Seed	100 mg	Lysing Matrix A	6.0	40 sec.
Oat FL 502	Leaf Tissue	75 mg	Lysing Matrix D	6.0	40 sec.
Oat FL 502	Seed	100 mg	Lysing Matrix A	6.0	40 sec.
Riser Oat	Leaf Tissue	70 mg	Lysing Matrix D	6.0	40 sec.
Richland Soybean	Leaf Tissue	100 mg	Lysing Matrix D	6.0	40 sec.
Tam Wheat	Leaf Tissue	75 mg	Lysing Matrix D	6.0	40 sec.
Tam Wheat	Root	80 mg	Lysing Matrix A	6.0	40 sec.
Tomato, Early Girl	Leaf Tissue	75 mg	Lysing Matrix D	6.0	4 x 30 sec.
Williams 82 Soybean	Leaf Tissue	70 mg	Lysing Matrix D	6.0	40 sec.
Wrens Rye	Leaf Tissue	100 mg	Lysing Matrix D	6.0	40 sec.
Pine	Needle	100 mg	Lysing Matrix A	6.0	30 sec.
BACTERIA					
Listeria monocytogenes	Cells	10^9 cells	Lysing Matrix B	6.0	3x 30 sec.
Streptococcus pyogenes	Cells	10^9 cells	Lysing Matrix B	6.0	20 sec.
Streptococcus mutans	Cells	10^9 cells	Lysing Matrix B	6.0	30 sec.
Staphylococcus aureus	Cells	10^8 cells	Lysing Matrix B	6.0	2x 40 sec.
Photorhabdus luminescens	Cells	10^9 cells	Lysing Matrix B	6.0	2x 30 sec.
Escherischia coli	Cells	10^8 cells	Lysing Matrix B	6.0	30 sec.
Mycobacterium tuberculosis	Cells	10^8 cells	Lysing Matrix B	6.0	2x 45 sec.
Lactococcus lactis	Cells	10^8 cells	Lysing Matrix B	6.0	3x 30 sec.
YEAST AND FUNGI					
Saccharomyces cerevisiae	Cells	2x 10^8 cells	Lysing Matrix C	6.0	40 sec.
Schizosaccharomyces pombe	Cells	10^8 cells	Lysing Matrix C	5.0	4x 15 sec.
Candida albicans	Cells	10^8 cells	Lysing Matrix C	6.0	2x 30 sec.
Cryptococcus neoformans	Cells	10^8 cells	Lysing Matrix C	6.0	4x 30 sec.
Aspergillus fumigatus	Cells	10^8 cells	Lysing Matrix C	6.0	2x 30 sec.
Fusarium solani	Cells	10^8 cells	Lysing Matrix C	6.0	2x 30 sec.

8. Recommended Reference Format for Publications

DNA was isolated from (specific sample) using the FastDNA® Green SPIN Kit and the FastPrep® Instrument (MP Biomedicals, Santa Ana,CA)

9. References

C.albicans -
Sergey V. Balashov et al. (2006). Antimicrob. Agents and Chemother. Vol 50: 2058-2063.

Fungi (P. boydii) -
Felix Gilgado et al. (2005). J.Clin.Microbiol. Vol 43: 1930-1942.

Fungi (S. schenckii) -
Rita Marimon et al. (2006). J. Clin. Microbiol., Vol 44: 3251 - 3256.

Plant seeds -
Els J.M. Van Damme et al. (2007). Plant Physiology, Vol 144 : 662-672.

Tomato leaves -
Hangsik Moon et al. (2004). Journal of Experimental Botany, Vol 55(402): 1519-1528.

Murine Intestine -
Alexandra J Scupham et al. (2006). Appl. Envir. Microbiol., Vol. 72: 793 - 801.

Brain -
Jean E. Jewell et al. (2005). J. Gen. Virol., Vol 86: 2127 - 2134.

Streptococcus pyogenes -
Audry C. Almengor et al. (2004). J. Bacteriol., Vol 186: 7847-7857.

Mycobacterium mucogenicum (Gram positive bacteria) -
Toïdi Adékambi et al. (2006). J. Clin. Microbiol., Vol 44: 837 - 840.

Blood -
Jonas Bunikis et al. (2004). J. Infectious Disease, Vol 189: 1515-1523.

10. Related Products

Description	Size	Catalog #
FastPrep® 24 Instrument	100-230V	6002-500
FastPrep® FP100A Instrument	100V	6001-100
FastPrep® FP120A Instrument	120V	6001-120
FastPrep® FP220A Instrument	220V	6001-220
FastDNA® Kit	100 preps	6540-400
FastDNA® SPIN Kit	100 preps	6540-600
FastDNA® SPIN Kit for Soil	50 preps	6560-200
FastRNA® Pro Soil-Direct Kit	50 preps	6070-050
FastRNA® Pro Soil-Indirect Kit	50 preps	6075-050
FastRNA® Pro Red Kit (Yeast & Fungus)	50 preps	6035-050
FastRNA® Pro Green Kit (Plant & Animal)	50 preps	6045-050
FastRNA® Pro Blue Kit (Bacteria)	50 preps	6025-050
FastProtein™ Blue Matrix	50 preps	6550-400
FastProtein™ Red Matrix	50 preps	6550-600
Lysing Matrix A	50 x 2 ml tubes	6910-050
Lysing Matrix A	100 x 2 ml tubes	6910-100
Lysing Matrix A	500 x 2 ml tubes	6910-500

11. 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

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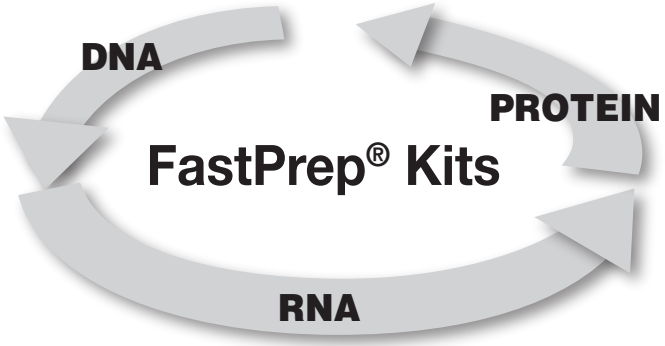
Take Advantage of FastPrep® Kits Ready-to-use Protocols For DNA, RNA And Protein Isolation From Any Sample

- Rapid and reproducible sample lysis and purification process
- No cross contamination with the closed lysing matrix tubes
- Increased yields of high quality DNA, RNA and proteins
- Integrity and size of DNA, RNA and proteins are retained
- Nucleic acids and proteins are ready-to-use in downstream application



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 Soil

Cat N° 6560-200 (100 preps)

- Lyse and isolate DNA in less than 30 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^{10} 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 10^{10} 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

Instruction Manual

FastDNA Green® SPIN

Protocol Revision # 6540-800-08FEB

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