MP Biomedicals

FastPrep 96 Operations Manual

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Section I Introduction

The FastPrep-96™ is a high-speed, benchtop reciprocating instrument for efficient disruption of cell membranes. The unit is designed for use with MP Biomedicals FastPrep Extraction Kits. These kits are available for a wide variety of extraction and purification applications. The FastPrep-96™ sample holder has the capacity to accommodate two 96-deep well plates simultaneously. We do not recommend using any lysing matrix plates other than those supplied by MP Biomedicals.

Different parts of FastPrep-96™ are shown below: (Fig. 1 & 2)

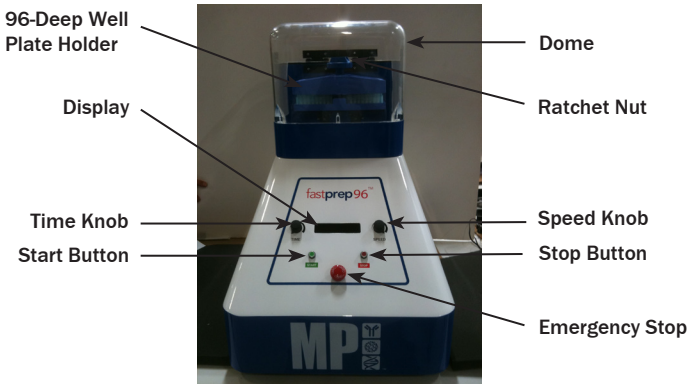


Figure 1

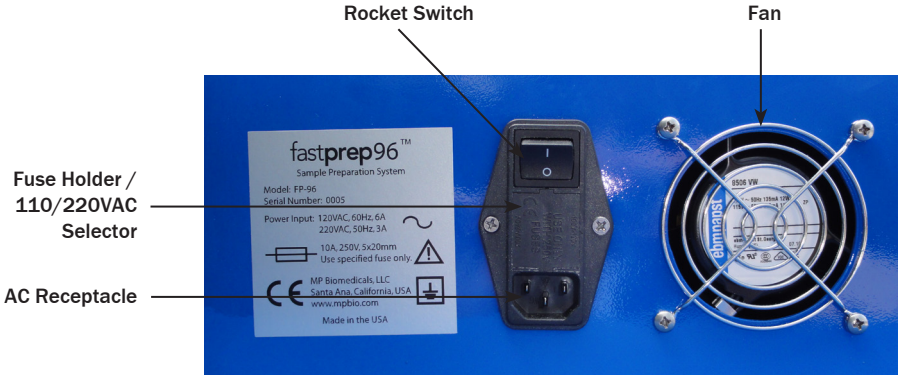


Figure 2

Section II Installation

2.0 Unpacking

Carefully remove the FastPrep-96™ instrument and accessories from the shipping crate by following these steps.

Note: It is recommended to retain all crating material (screws, foam, crate panels etc.) as this packaging is safest way to transport the instrument. Transport in other packaging may void the product warrantee.

1. Using a standard Phillips screwdriver or powerdriver, carefully remove screws securing top of crate.

2. Remove the crate top

3. Unscrew the attachments that secure the restraining bar on one of the side panels. (Figure 3)

4. On that same side panel, remove the screws securing that panel and remove the panel.

5. Remove the restraining bar screws on the opposite side panel.

6. Remove the restraining bar carefully as to not damage the machine surface.

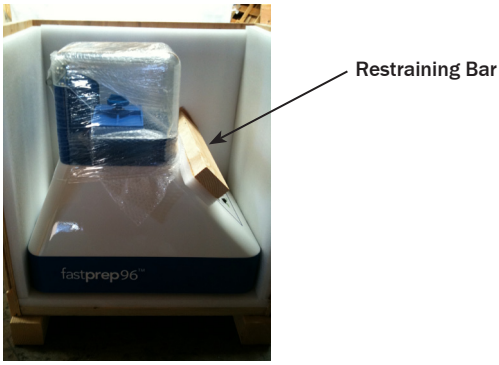


Figure 3

7. Remove the instrument carefully and place it on a sturdy table or bench top.

8. Position the instrument such that ~10 centimeters of the front of the instrument overhangs from the edge of the bench top. This will expose the lower transport wing screw that must be removed (Figure 4).

VERY IMPORTANT INSTRUCTION!

9. Unscrew and remove the temporary transport wing screw (Figure 4). Once removed, reposition machine and remove rear screw which is identical (Figure 5). There are two mounting screws, one in the front and one in the rear.

IMPORTANT! Both of these temporary transport screws MUST BE REMOVED PRIOR TO OPERATION. Failure to do so could result in damage to the instrument or personal injury!

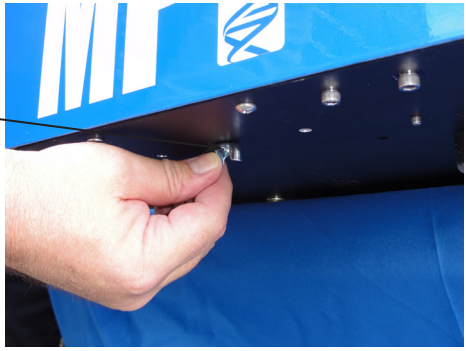


Figure 4

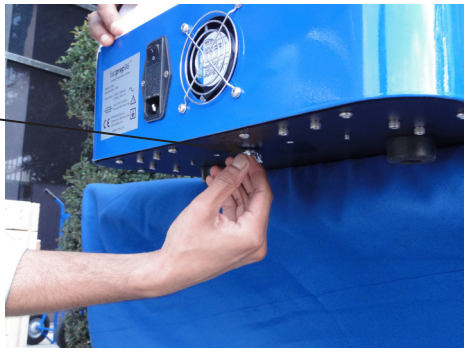


Figure 5

10. Re-position the instrument on table or bench top. Place the system on a clean, dry-stable surface within 4 feet (1.2m) of a compatible electrical outlet.

11. Remove remaining packaging foam from upper dome assembly

2.1 Inspection

Inspect the unit for any damage that may have occurred during shipment. Should there be any damage, report it to the carrier and contact MP Biomedicals immediately. Save the packaging material in the event a return is necessary.

FastPrep-96™ comes complete with all the necessary accessories to run the instrument.

Items included are listed below.
1. 3-pin AC Cord Flat - 1
2. Fuse 10 Amp - 2
3. Instruction Manual – 1
4. 96 deep well plate holder with ratchet nut - 1

Compare the packing list to the box contents. If there is a discrepancy, please contact MP Biomedicals

Ventilation
Allow 1-2 inches (3-5 cm) of space around the FastPrep-96™ instrument for proper ventilation. This unit is "FOR INDOOR USE ONLY". Avoid operating in areas of excessive humidity or temperature extremes.

2.2 Set-up, Controls and Functions

To assure safe operation and best results, read this manual before operating the FastPrep-96TM instrument. TheFastPrep-96™ instrument comes fully assembled, requiring very little set-up. Install the system on a clean, dry-stable surface within 4 feet (1.2m) of a compatible electrical outlet.

The function of the control panel keys are listed below (Ref. Fig. 6)
1. Speed knob – Turn knob to set speed in revolutions per minute (RPM).
   Speed & Time as shownon display
   Speed: Selectable from 800 RPM to 1800 RPM in increments of 100 RPM
   (Default is 800 RPM).
2. Time knob – Selectable from 1 sec. to 60 sec.in increments of 1 second. From 60 sec. to 360 sec in increments of 30 sec.
   (Default 2 is 0 seconds)
3. Start Button – starts the instrument.
4. Stop Button – stops the instrument.
5. Emergency Stop – cuts all power to instrument.

Fig.6 (Control Panel)

VERY IMPORTANT INSTRUCTION!

2.3 Connecting the Power

The FastPrep-96™ operates on 110 VAC/60 Hz or 230 VAC/50 Hz.
Important! The FastPrep-96™ is shipped in the configuration for 110 VAC/60 Hz (Figure 7). If using with 230VAC/50 Hz power supply (European) the fuse assembly must be re-oriented. To configure for 230VAC/50 Hz, use a screw driver to pry out the fuse assembly, invert 180 degrees, and re-install. (Figure 6). The indicator should point to the "200-240V" position (Figure 8).

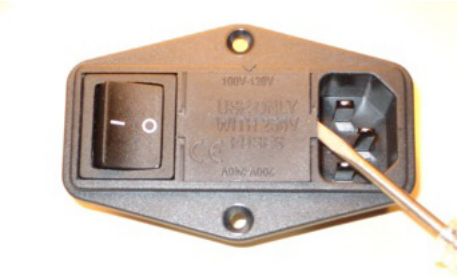


Figure 7 (shown in 110VAC/60 Hz configuration)

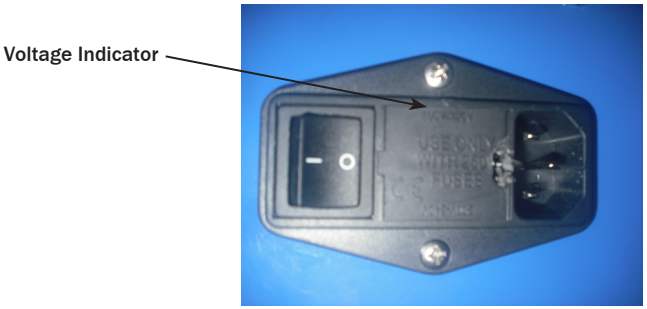


Figure 8 (shown in 230VAC/50 Hz configuration)

Make sure the rocker switch located on the rear panel is OFF when connecting the power. Connect the power cord to the instrument (AC receptacle is at the back of the instrument) and plug it in to a compatible outlet.

This symbolizes Alternating Current 120/240V ~

13. Safely secure 96 well plate adapter by making sure that the ratchet nut clicks.
14. Run machine at low RPM setting (800RPM)

IMPORTANT NOTE: If at any time, loud noises, grinding or whining noises occur, immediatley engage the EMERGENCY STOP BUTTON. This will immediately cut power and stop the instrument. Contact MP Biomedicals.

15. If low RPM run is OK, increase settings and run at 1200 RPM, then at 1800 RPM to verify. Recommended setting is 60 seconds for verification run time.

16. It is recommended that all packaging material be saved (screws, crate, packing material, and transport wing screws) in case instrument is to be transported again.

Section III Operation

3.0 LOADING & SECURING THE SAMPLES

1. Lift up dome to open position
2. Remove the securing knob (ratchet nut) by rotating counter-clockwise
3. Remove Top plate of 96 deep well plate holder. (Ref Fig 9)

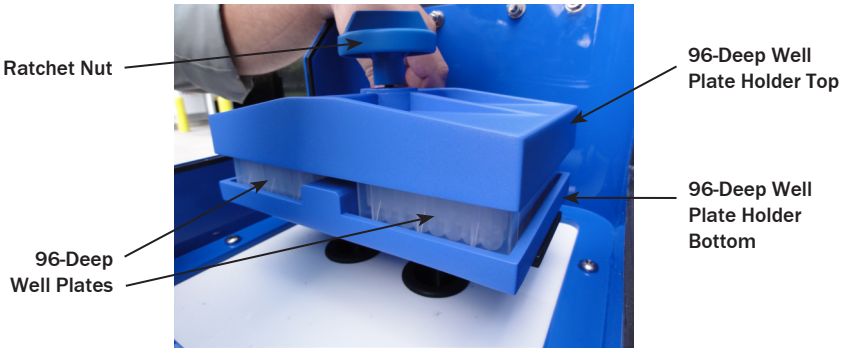


Figure 9

4. Load two (2) Lysing Matrix 96 well plates into the cavities of the sample holder so that they fit snugly. The sample holder must be balanced, so if only processing one (1) Lysing Matrix plate, load another blank plate in the other cavity.
5. With the Lysing Matrix plates in place, check that there is a snug fit. Replace the top plate of the sample holder, ensuring that the threaded hold-down rod is properly aligned and protrudes through the plate holder top. Squeeze top and bottom sample holder plates together with two hands to ensure a snug fit. (Ref Fig. 10)

Figure 10

6. Place the securing knob (ratchet nut) on this assembly to tighten it completely until you hear the ratchet click.

7. Close the dome

IMPORTANT NOTE: The dome contains an electro-magnetic safety over-ride. If the dome is not properly closed, the motor will not start.

 **WARNING**: Sample Lysing Plates must be secured properly before running the FastPrep-96™ instrument.

3.1 PREPARING THE FastPrep™-96 FOR OPERATION

1. Turn the main rocker switch to the ON position.
2. When unit is turned on, the display will be lit.
3. Factory-set default values automatically program FastPrep-96 to operate at a speed of 800 RPM and run time of 20 seconds.

3.2 PROGRAMMING THE FastPrep-96™

1. Adjust Speed Dial to desired RPM.
   Note: Speed is adjustable in from 800 to 1800 RPM in increments of 100
2. Adjust the time dial to the desired run time in seconds
   Note: Time is adjustable from 0 to 60 sec in increments of 1 sec, and from 60 to 360 sec in increments of 30 sec
3. Press start button to begin cycle. The motor will start and display will show count down run time.

    **WARNING**: Insure that adapter, well plates, and ratchet nut are securely fastened before running.
4. When the time is over, FastPrep-96™ will stop automatically and the program will go back to the main menu with the last selections made
5. Press the stop button to stop the instrument at any time. Resume programmed cycle by pressing the start button again. The previously entered program will resume from the point at which the run was stopped. Alternately, if the dome lid is opened at any time during a run, the program will stop immediately. Closing the Dome lid will re-start the run from the point of interruption. The program will run to completion.
6. Turn off the instrument by pressing the rocker switch to the OFF position.

 **CAUTION**:

1. Improper closing of dome will cause power interruption.
2. Do not run the machine without the sample holder, as this may cause damage to the motor shaft.
3. The instrument should be properly grounded for safe use.
4. Disconnect device from the socket in the event of danger.

Earth terminal 

IV Specifications

Controls: Programmable run time and speed; display readout
Time: Range: 1-360 Sec
Programmable 1-60 sec in 1 sec increments and 60-360 sec in 30 sec increments
Speed: Range: 800-1800 revolutions per minute (RPM)
Programmable in 200 RPM increments
Acceleration: <2 seconds to maximum speed
Deceleration: <2 seconds to stop
Weight: 49 kg
Power Requirement: 110VAC/60Hz, 5.2A or 220 VAC50Hz, 2.6A
Recommended operating temperature range: 35-100°F (2-48°C)
Recommended operating relative humidity range: 30-55%
Dimensions: 44cm wide x 66cm deep x 70cm high
Overvoltage Category: II
Maximum sound level: <70 dB
Maximum altitude: 2000 meters

V Symbols and Descriptions

Symbol	Description
	Alternating Current
	Earth Terminal, Ground
	Fuse
	Electrical Equipment, Dispose Properly
	Caution, Warning
	Biological Risk
	Power On
	Power Off

VI WARRANTY & LIABILITY

The FastPrep-96™ instrument is warranted against defects in material and workmanship for one year after the date of delivery to the original purchaser. This warranty is limited to defects in materials and workmanship, and does not cover incidental or consequential damages.

MP Biomedicals will repair free of charge any apparatus covered by this warranty. Warranty includes one-year parts and labor in our facilities or by approved distributors. Warranty work is subject to our inspection of the unit. No instruments, equipment, or accessories will be accepted without a Return Material Authorization (RMA) number issued by MP Biomedicals. Costs of shipping the unit are not covered under this warranty. The warranty obliges you to follow all precautions in this manual.

When returning an instrument that may contain hazardous and/or infectious materials, you must pack and label it according to US Department of Transportation (DOT) and / or European community (EC) regulations applying to transportation of hazardous and/or infectious materials. Your shipping documents must also meet DOT and/or EC regulations. All returned units must be fully decontaminated of any chemical, biological or infectious agents.

Use of this equipment in a manner other than those specified in this manual may jeopardize personal safety. Under no circumstances shall MP Biomedicals be liable for damages due to the improper handling, abuse, or unauthorized repair of these products. MP Biomedicals assumes no liability, expressed or implied, for use of this equipment.

Use of non-approved kits and reagents with the FastPrep-96™ instrument is not covered under this warranty.

VII APPENDICES

APPENDIX 1

MAINTENANCE & CLEANING

Maintenance: The FastPrep-96™ instrument requires no scheduled maintenance. Clean surfaces immediately after contact with sample solutions or reagents.
Remove sample holder to wipe inner surfaces. (Refer to Fig. 9)

Cleaning: The Fastprep-96™ instrument should be cleaned if reagents or sample solutions spill on or inside the unit. If a sample tube leaks during a run, the solution will be sprayed on the bowl. Always clean up any spray or spills immediately using a damp paper towel. Always wear gloves and protective clothing when cleaning. If potentially infectious agents are used in the FastPrep-96™ instrument, spills should be cleaned immediately, and appropriate decontamination carried out. The FastPrep-96™ instrument may not be resistant to all cleaning regimens required for all infectious agents. Exercise appropriate caution and wear protective clothing, eyewear and gloves when working with potentially infectious samples. Contaminated units should be kept in an appropriate level biosafety facility, and should only be maintained or serviced by personnel trained in safe handling practices specific to the infectious agent.
(See Appedix V for information about MP Biomedicals 7X™ line of Cleaning Solutions)

Fuse Replacement:

Caution: Disconnect input power before replacing fuse. For continued fire protection replace fuse only with the specified type and appropriate rating. Fuse rating is 10 Amp (T10AL250V). 2 extra fuses are provided with the accessories. If a fuse blows, follow the steps below for replacement (Ref. Fig. 7)

1. Ensure that input power is disconnected during replacement of fuse.
2. Take out fuse holder from the AC receptacle provided at the back side of the main instrument. A spare fuse is provided in the fuse holder. Remove the faulty fuse and replace it with new one.
3. Insert the fuse holder back into the AC receptacle carefully.
4. Reconnect the power cord to the instrument and plug it into a compatible outlet and turn on the power switch.

APPENDIX 2

AN EXPLANATION OF FastPrep-96™ INSTRUMENT SPEED SETTINGS

The cell disruption process during a FastPrep-96™ instrument run is caused by the collision of matrix and sample within the FastPrep-96™ instrument sample well. The rate of collision and energy of impact (both of which determine the effectiveness of the disruption process) are a function of the FastPrep-96™ instrument speed settings and specific gravity of the bead material used. The FastPrep-96™ instrument speed settings in rpm refer to the maximum vertical velocity achieved by a sample tube during reciprocating motion. The rate of collision is proportional to speed, while the energy of impact is proportional to the square of the speed. For example, a 50% increase in the FastPrep®-96 instrument speed setting will increase the rate of collision by 50% and at the same time increase the energy of impact by 125%. The FastPrep-96™ instrument has been specifically designed to allow operation within an ideal range of parameters for disrupting membranes from a wide variety of cell types. When used with cell-specific protocols and kits from MP Biomedicals, cell membrane disruption and nucleic acid yield is maximized.

APPENDIX 3

THE FastPrep-96™ PRODUCT LINE

FastDNA-96™ Soil Microbe DNA Kit

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

The FastDNA-96™ Soil Microbe DNA Kit for FastPrep-96™ is designed for the simple and rapid isolation of humic-free, PCR-quality DNA from microbes in soil. This kit can be used to isolate DNA from tough-to-lyse bacteria, fungi, protozoa, and algae that inhabit a variety of samples including clay, sandy, silty, peaty, chalky, and loamy soils. First, soil microbes are rapidly and efficiently lysed by bead beating using specialized lysing matrix particles and the FastPrep-96™ instrument. Then spin column plate technology is used to isolate the DNA, which is subsequently filtered to remove humic acids/polyphenols that can inhibit PCR. This procedure can be performed in minutes, and there is no need for organic denaturants or proteinases.

Detailed Protocol

1. To the tubes of a **FastDNA-96™ Lysing Matrix Rack**, add up to 135 mg of soil and 400 µl of **Lysis Buffer** per well. Re-cap the tubes.

Optional: If broth culture cell lysis is desired, add 10-20 mg of wet weight bacterial or fungal cells, resuspended in up to 50 µl of sterile water, or sterile PBS, to the tubes of the **FastDNA-96™ Lysing Matrix Rack**. Add **Lysis Buffer** and continue.

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 to 10 - 15 minutes can enhance elimination of excessive debris from soil samples, or from cells with complex cell walls.

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

5. Add 750 µl of **Soil 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 µl 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 **Soil DNA Wash Buffer**. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x *g*.

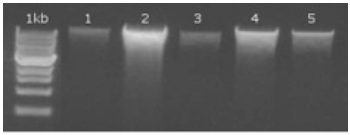
11. Stack the **MP-96 Binding Plate** atop a clean **Elution Plate**. Add 50 - 100 µl of **Elution Buffer** directly to the matrix inside the wells of the **MP-96 Binding Plate**. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x *g*.

NOTE: DNA isolation is complete at this point if bacterial of fungal broth cultures were sampled. Continue with protocol only if sampling from soil or sludge.

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.

Example Data: DNA Isolation from Soil Samples



DNA from various soil samples extracted with the FastDNA-96™ Soil Microbe Kit. 10% of the DNA isolated from 135 mg soil samples was loaded on a 0.8% agarose/ ethidium bromide gel. (1 kb Ladder Marker)
Soil samples include: Lane 1: Sand; Lane 2: Sandy Clay; Lane 3: Sandy Loam; Lane 4: Coarse Sandy Loam; Lane 5: Fine Gravel Sandy Loam. DNA ranges from 4-20 kb

Figure 1.

2 x 96 Preps
Storage: Ambient temperature (15 – 30°C)
Catalog # 9696-200

FastDNA-96™ Fungal & Bacterial DNA Kit

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

The FastDNA-96™ Fungal/Bacterial DNA Kit for FastPrep-96™ is designed for the simple and rapid isolation of DNA from tough-to-lyse fungi, including *A. fumigatus, C. albicans, N. crassa, S. cerevisiae, S. pombe*, as well as Gram (+/-) bacteria, algae, and protozoa. The procedure is easy and can be completed in minutes: fungal and/or bacterial samples are rapidly and efficiently lysed by bead beating using specialized lysing matrix particles and the FastPrep-96™ instrument. Then spin column plate technology is used to isolate the DNA for downstream molecular-based applications including PCR, array, etc.

Detailed Protocol

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 400 µl 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: $2x10^8$ bacterial cells, $2x10^7$ yeast cells, or $2x10^6$ 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.

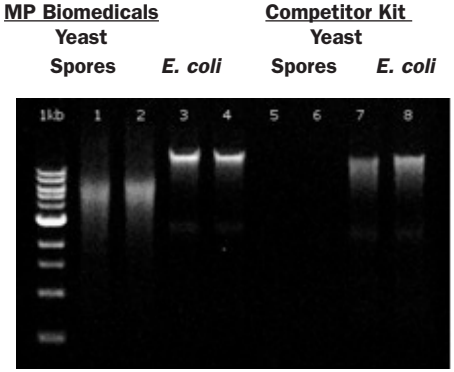
9. Continue to re-use the **Collection Plate** by placing it beneath the **MP-96 Binding Plate**. To the wells of the **MP-96 Binding Plate**, add 200 µl of the **Binding Plate Pre-Wash Buffer**. Centrifuge the stacked plates for 5 minutes at 3,500 - 5,000 x *g*.

10. To the wells of the **MP-96 Binding Plate**, add 500 µl of **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** (25 µl 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.

Example Data: DNA Isolation from Bacteria & Yeast Samples



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).

Figure 2.

2 x 96 Preps
Storage: Ambient temperature (15 – 30°C)
Catalog # 9696-300

FastDNA-96™ Fecal DNA Kit

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

The FastDNA-96™ Fecal DNA Kit for FastPrep-96™ is designed for the simple and rapid isolation of inhibitor-free, PCR-quality host cell and microbial DNA from a variety of sample sources including humans, birds, rats, mice, cattle, etc. The procedure is easy and can be completed in minutes: fecal samples are rapidly and efficiently lysed by bead beating using specialized lysing matrix particles and the FastPrep-96™ instrument. Spin column plate technology is then used to isolate the DNA which is subsequently filtered to remove humic acids/polyphenols that can inhibit PCR. Eluted DNA is ideal for downstream molecular-based applications including PCR, arrays, genotyping and methylation detection.

Detailed Protocol

1. To the tubes of a **FastDNA-96™ Lysing Matrix Rack**, add up to 80 mg of wet or dry feces and 400 ml 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 **Fecal 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 µl of the **Binding Plate Pre-Wash Buffer**. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x *g*.

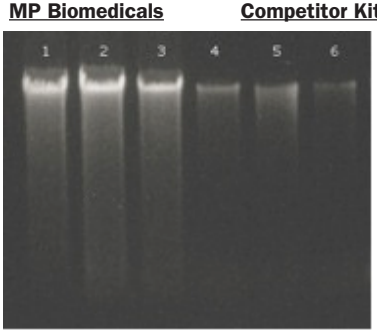
10. To the wells of the **MP-96 Binding Plate**, add 500 µl of **Fecal DNA Wash Buffer**. Centrifuge the stacked plates for 5 minutes at 3,500 -5,000 x *g*.

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 3.3 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.

Example Data: DNA Isolation from Fecal Samples



Comparison of DNA purified from rat feces using the FastDNA-96™ Fecal DNA Kit and a competitor kit. DNA was isolated from 80 mg stool samples and was loaded on a 0.8% agarose/ethidium bromide gel.

Figure 3.

2 x 96 Preps
Storage: Ambient temperature (15 – 30°C)
Catalog # 9696-400

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

The FastDNA-96™ Tissue & Insect DNA Kit for FastPrep-96™ is designed for the simple and rapid isolation of DNA (e.g., genomic, viral, mitochondrial) from fresh, frozen, or stored insect specimens including mosquitoes, bees, lice, ticks, and D. melanogaster. The procedure is easy and can be completed in minutes: samples are rapidly and efficiently lysed by bead beating using specialized lysing matrix particles and the FastPrep-96™ instrument. The DNA is then isolated and purified using spin column plate technologies. The DNA is now ideal for downstream molecular-based applications including PCR, array, genotyping, etc. This procedure is also compatible with mammalian tissues, whole blood, and cultured cells.

Detailed Protocol

1. To the tubes of a **FastDNA-96™ Lysing Matrix Rack**, add 1-10 mg of fresh or frozen mammalian or insect tissue, $1.7x10^6$ 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™ 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 **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 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.

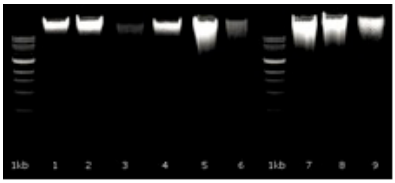
9. Continue to re-use the **Collection Plate** by placing it beneath the **MP-96 Binding Plate**. To the wells of the **MP-96 Binding Plate**, add 200 µl of the **Binding Plate Pre-Wash Buffer**. Centrifuge the stacked plates for 5 minutes at 3,500 - 5,000 x *g*.

10. To the wells of the **MP-96 Binding Plate**, add 500 ml 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 µl of Elution Buffer (25 µl 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.

Example Data: DNA Isolation from Tissue & Insect Samples



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).

Figure 4. 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.

Figure 4.

2 x 96 Preps
Storage: Ambient temperature (15 – 30°C)
Catalog # 9696-500

FastDNA-96™ Plant & Seed DNA Kit

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

The FastDNA-96™ Plant/Seed DNA Kit for FastPrep-96™ is designed for the simple, rapid isolation of inhibitor-free, PCR-quality DNA from a variety of plant sample sources including leaves, stems, buds, flowers, fruit, and seeds. The procedure is quick and easy: plant samples are rapidly and efficiently lysed by bead beating using specialized lysing matrix particles and the FastPrep-96™ instrument. Then the polysaccharides, lipids, and polyphenols/tannins are removed from the DNA using spin column plate technology. The eluted DNA is filtered to remove polyphenolics, making it ideal for downstream molecular-based applications including PCR, arrays, etc.

Detailed Protocol

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 µl 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.

Example Data: DNA Isolation from Plant & Seed Samples

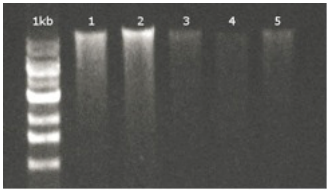


Figure 5a.

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)

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

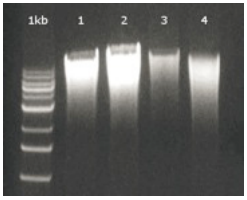


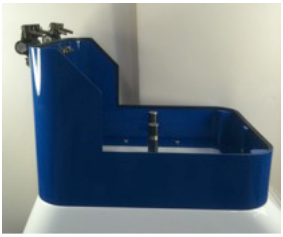
Figure 5b.

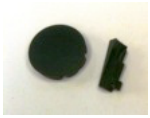
Figure 5b. samples include:
Lane 1-2: Corn Kernel; Lane 3-4: Sunflower Seed

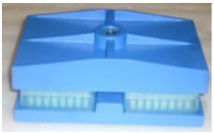
2 x 96 Preps
Storage: Ambient temperature (15 – 30°C)
Catalog # 9696-600

APPENDIX 4

ACCESSORIES AND SPARE PARTS FOR FastPrep-96™

Chamber Assembly CAT#119696111 (Includes) Chamber Bottom, CAT#119696112 Chamber Top, CAT#119696115 Magnet, CAT#119696113 Sensor, CAT#119696114 Gasket, CAT#119696117 Chamber Hinge, CAT#119696119	
Chamber Bottom, CAT#119696112	
Chamber Top CAT#119696115	
Chamber Hinge CAT#119696119	
Start button CAT#119696151	

Stop button CAT#119696143	
Time/Speed potentiometer, knob assembly: CAT#119696155	
Potentiometer Knob CAT#119696153	
Potentiometer Marker CAT#119696154	
Potentiometer Cover CAT#119696122	
Emergency Stop Button CAT#119696228	
Ratchet Nut CAT#119696161	

Dual Plate Holder Assembly CAT#119696168	
6' Power Cord CAT#119696166	

APPENDIX 5

CLEANING RECOMMENDATIONS, 7X™ CLEANING SOLUTION

 FastPrep-96™ Cleaning 

To avoid possible damage to delicate instrument surfaces, such as gel coat finish and labels, and to avoid corrosion of metal parts, and crazing of transparent lid, the preferred method for cleaning FastPrep-96™ is with MP Biomedicals 7X™ Brand Cleaning Solution. Since 1950, 7X™ has been a safe and effective cleaning solution for many laboratory applications.

The composition of 7X™ is a proprietary mixture of a strong, phosphate-free sequestering reagent, a completely-soluble and non-toxic solvent, with no-residue leftovers after evaporation, and powerful, environmentally friendly wetting/foaming/emulsifying agents. This combination, along with a powerful anionic surfactants makes 7X™ an ideal solution for applications which require low cell toxicity, low debris, high efficiency cleaning, and non-corrosion on the cleaned substrates, especially glass. Furthermore, it does not contain any fluorescent components. As such, 7X™ laboratory detergent is ideal for thorough cleaning of sensitive equipment such as FastPrep-96™, bioreactors, cultureware and general lab equipment including test tubes, microscopy slides, pathology equipment, Petri dishes, culture flasks and cylinders, burets, pipettes, slide covers, and hypodermic syringes as well as optical components due to absence of fluorescence. Furthermore, it is widely used in industrial applications for cleaning of chemical and bioreactors and fermentors and is preferred method for cleaning glassware and plasticware for human *in vitro* fertilization.

Soluble in water at any concentration, 7X™ drains completely from glassware in only a few seconds. A 5% solution in distilled water has a pH range of 6–9. This neutral pH will not induce or cause etching of delicate plastics, metal and glass surfaces and will not leave any residues.

7X™ is effective at temperatures ranging from ambient to 85°C (185°F). Due to its unique properties, 7X™ has been widely used and publicized, with almost 8,000 peer-reviewed publications citing the use of 7X™, as indexed on http://scholar.google.com, search term "7X detergent".

7X™ CLEANING SOLUTIONS

Regular 7X™

This powerful solution was designed to clean laboratory plastics and glassware simply by soaking overnight. You may boil equipment in 7X. This products surfactants are extremely effective and powerful.

CAT#097667049	100 ml Sample Size
CAT#097667093	1 gallon Plastic Bottle
CAT#097667094	4 x 1 Gallon Case
CAT#097667095	1 x 5 gallon

ES-7X™

"Environmentally Safe" phosphate-free ES works just like the original &X and is designed to work in soaking (non-agitated) applications. It is highly concentrated and goes a long way.

CAT#097667149	100 ml Sample Size
CAT#097667193	1 gallon Plastic Bottle
CAT#097667194	4 x 1 Gallon Case
CAT#097667195	1 x 5 gallon

Regular 7X™ Ready-to-Use

5% solution of the classic 7X, pre-diluted to a convenient working solution. Includes a hand pump for easy dispensing.

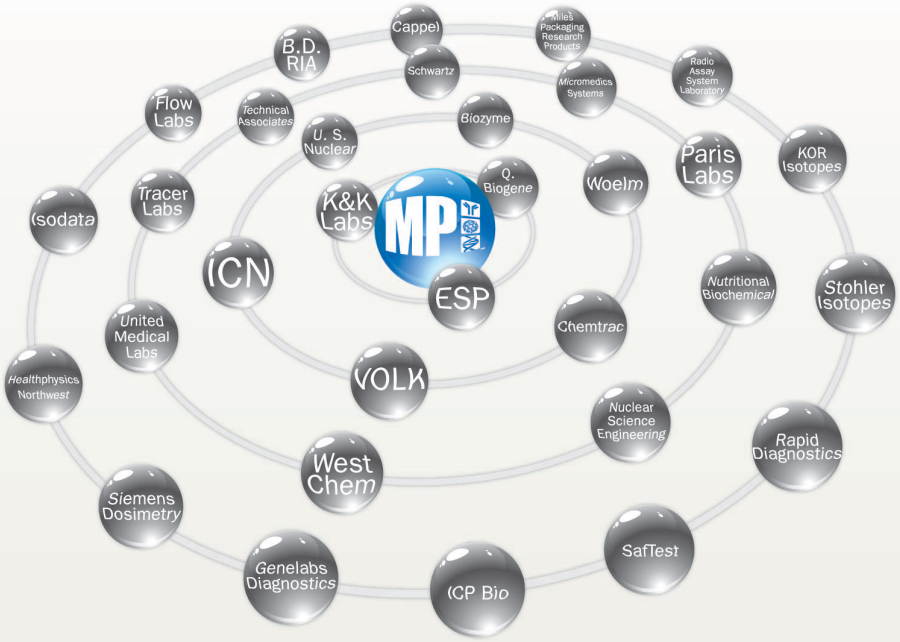
CAT#097668093	1 gallon Plastic Bottle

ES-7X™ Ready-to-Use

5% solution of the ES-7X, pre-diluted to a convenient working solution. Includes a hand pump for easy dispensing.

CAT#097667193	1 gallon Plastic Bottle

A Constellation of Quality Products



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