PowerPlant® DNA Isolation Kit

Catalog No.	Quantity
13200-50	50 Preps
13200-100	100 Preps

Instruction Manual

New protocol instruction: *Shake Solution PB4 to mix before each use to ensure consistent results.*

F **Please recycle**

Version 08202010

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Introduction

The PowerPlant® DNA Isolation Kit provides a novel method for isolating genomic DNA with a high level of purity, allowing for successful PCR amplifications from a variety of plant samples.

Plant samples are added to a bead tube containing 4 stainless steel beads along with a kit supplied buffer for rapid homogenization. Cell lysis and DNA release occurs by mechanical and chemical methods. Released genomic DNA is first precipitated with isopropanol and then captured on a silica membrane in a spin column format. DNA is washed and eluted from the membrane and ready for PCR and other downstream applications. The PowerPlant® DNA Isolation Kit provides PCR ready DNA from a wide variety of plant leaves, roots, and seeds.

The PowerPlant® DNA Isolation Kit also comes with extra volumes of reagents that can be used to perform a simple DNA clean-up protocol. This may be required for DNA from plants high in polysaccharides or phenolics to improve its functional quality. Occasionally, plants such as grapes, cotton, sunflower, strawberry, pine needles, etc. will yield DNA with inhibitors, which may prevent target sequences from amplifying in PCR. Under such circumstances, it is suggested to use the clean-up protocol provided. Alternatively you can dilute the template DNA one to several fold, for successful PCR.

Mechanical Lysis Options

The PowerPlant® DNA Isolation Kit may be used with the vortex or the high velocity bead beater, PowerLyzer™ 24 homogenizer. The PowerLyzer™ 24 is suitable for fast homogenization of plant materials including stems, roots, seeds, or difficult leaf tissue.

PowerLyzer™ 24
Bench Top Bead-Based Homogenizer
Catalog#13155
(www.mobio.com/powerlyzer)

Using the PowerPlant® DNA Isolation Kit with the PowerLyzer™ Homogenizer

The PowerLyzer™ 24 is a highly efficient bead beating system that allows for optimal DNA extraction from a variety of plant tissues. The instrument's velocity and proprietary motion combine to provide the fastest homogenization time possible, minimizing the time spent processing samples. The programmable display allows for hands-free, walk-away extraction with up to ten cycles of bead beating for as long as 5 minutes per cycle. This kit provides Bead Tubes prefilled with 2.38 mm stainless steel beads for homogenizing plant tissue for optimal DNA isolation. Alternative pre-filled bead tube options are available for additional applications. Please contact technical service (technical@mobio.com) for details.

For isolation of DNA from plant tissues using this kit with the PowerLyzer™, guidelines for getting started can be found in step 6 on page 7 of the protocol.

Using the PowerPlant® DNA Isolation Kit with other Homogenizers

For isolation of DNA using this kit with the FastPrep® or Precellys®, the following conversion chart will help you to adapt your current protocol. However, due to the highly efficient motion of beads in the PowerLyzer™ 24, we have found that less cycle numbers are required to generate the same effect. You may want to perform extractions on the PowerLyzer™ 24 at the equivalent speed and number of cycles as your current instrument and compare it to less time or lower speed to determine which settings give the best results.

PowerLyzer 24	Fastprep 24 m/s	Precellys 24
500	-	-
600	-	-
700	-	-
800	-	-
900	-	-
1000	-	-
1100	-	-
1200	-	-
1300	-	-
1400	-	-
1500	-	-
1600	-	-
1700	-	-
1800	-	-
1900	-	-
2000	-	-
2100	-	-
2200	-	-
2300	-	-
2400	-	-
2500	4	5000
2600	-	5200
2700	-	5400
2800	4.5	5600
2900	-	5800
3000	-	6000
3100	5	6200
3200	-	6400
3300	-	6600
3400	5.5	6800
3500	-	-
3600	-	-
3700	6	-
3800	-	-
3900	-	-
4000	6.5	-
4100	-	-
4200	-	-
4300	-	-
4400	-	-
4500	-	-
5000	-	-

Equivalent settings slower than 2500 RPM or higher than 4000 RPM on the PowerLyzer™ 24 are not obtainable with the Fastprep® or Precellys®.

Fastprep® is a registered trademark of MP Biomedical. Precellys® is a registered trademark of Bertin Technologies.

High Throughput Options

MO BIO offers a vacuum based protocol for faster processing without centrifugation for the DNA binding and column washing steps for Spin Filters. The MO BIO PowerVac™ Manifold allows for processing of up to 20 spin filter preps at a time using the PowerVac™ Mini Spin Filter Adapters. For additional high throughput options MO BIO offers the UltraClean®-htp 96 Well Plant DNA Isolation Kit for processing up to 2 x 96 samples using a centrifuge capable of spinning two 96 Well Blocks stacked (13 cm x 8 cm x 5.5 cm) at 2500 x *g.* For 96 well homogenization of plant tissue, MO BIO offers the 96 Well Plate Shaker and Plate Adapter Set (MO BIO Catalog# 11996 & 11999, respectively.)

This kit is for research purposes only. Not for diagnostic use.

Other Related Products	Catalog No.	Quantity
UltraClean® Plant RNA Isolation Kit	13300-20 13300-50	20 preps 50 preps
UltraClean®-htp 96 Well Plant DNA Isolation Kit	13096-4 13096-12	4 x 96 preps 12 x 96 preps
UltraClean® 15 DNA Purification Kit	12100-300	300 preps
PowerLyzer™ 24 homogenizer	13155	1 unit
Vortex Adapter, holds 24 (1.5-2.0 ml) tubes	13000-V1-24	1 unit

Equipment Required

Centrifuge for 2ml tubes (13,000 x *g*)
Pipettor (50 µl – 200 µl, 100 µl – 1000 µl)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
Vortex Adapter (MO BIO Catalog# 13000-V1)

Kit Contents

	Kit Catalog# 13200-50		Kit Catalog# 13200-100	
Component	**Catalog #**	**Amount**	**Catalog #**	**Amount**
PowerPlant® Bead Tubes	13200-50-BT	50	13200-100-BT	100
PowerPlant® Bead Solution	13200-50-BS	35 ml	13200-100-BS	70 ml
PowerPlant® Solution PB1	13200-50-1	5.5 ml	13200-100-1	11 ml
PowerPlant® Solution PB2	13200-50-2	20 ml	13200-100-2	43 ml
PowerPlant® Solution PB3	13200-50-3	3 x 30 ml	13200-100-3	6 x 30 ml
PowerPlant® Solution PB4	13200-50-4	28 ml	13200-100-4	55 ml
PowerPlant® Solution PB5	13200-50-5	30 ml	13200-100-5	2 x 30 ml
PowerPlant® Solution PB6	13200-50-6	11 ml	13200-100-6	22 ml
PowerPlant® Solution PB7 (for use with clean-up protocol only)	13200-50-7	4 ml	13200-100-7	8 ml
PowerPlant® Spin Filters	13200-50-SF	50	13200-100-SF	100
PowerPlant® 2 ml Collection Tubes	13200-50-T1	100	13200-100-T1	200
PowerPlant® 2.2 ml Collection Tubes	13200-50-T2	50	13200-100-T2	100

Kit Storage

Kit reagents and components should be stored at room temperature (15-30 °C).

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: Solution PB3 is 99% isopropanol and Solution PB5 contains ethanol. Both of these reagents are flammable.

CAUTION: The integrity of the PowerPlant® Bead Tubes is guaranteed only if they are used within the parameters outlined in this protocol. Exceeding these parameters may result in cracked or damaged tubes.

IMPORTANT NOTE FOR USE: Make sure the 2 ml PowerPlant® Bead Tubes rotate freely in your centrifuge without rubbing. Shake to mix Solution PB4 before each use.

Experienced User Protocol

Please wear gloves at all times

1. To the PowerPlant® Bead Tubes provided, add up to 50 mg (0.05 g) of plant tissue sample, followed by the addition of 550 µl of PowerPlant® Bead Solution.
   NOTE: THE DNA EXTRACTION EFFICIENCY WILL IMPROVE BASED ON THE CONDITION OF HOMOGENIZATION AND THE TYPE OF HOMOGENIZATION METHOD. PLEASE REFER TO THE "ADDITIONAL INFORMATION" SECTION FOR DIFFICULT PLANT TYPES.
2. Gently vortex to mix.
3. **Check Solution PB1**. If Solution PB1 has precipitated, heat solution to 60°C until dissolved before use.
4. Add 60 µl of Solution PB1 and invert several times or vortex briefly.
5. Place the PowerPlant® Bead Tubes in a water bath at 65°C for 10 minutes.
6. Homogenize using one of the following methods:

   A. Vortex:
   Secure PowerPlant® Bead Tubes horizontally using the MO BIO Vortex Adapter (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

   B. PowerLyzer™ Homogenizer:
      1. Properly identify each PowerPlant® Bead Tubes on both the cap and on the side.

   Note: Due to the high energies of the PowerLyzer™ 24, the potential of marring of the tops of the caps is possible, therefore it is recommended to mark the sides of the PowerPlant® Bead Tubes as well as the caps to ensure proper sample identification.

      2. Place Bead Tubes into the Tube Holder of the PowerLyzer™ 24. The Bead Tubes must be balanced (evenly spaced) on the Tube Holder. Homogenize the tissue for 1 cycle at the chosen speed depending on your sample type for 3 minutes.

Plant Tissue Type	Speed	No. of Cycles	Time/Cycle
Soft leaf tissues	2000 RPM	1	3 minutes
Fibrous leaf tissues	2200 RPM	1	3 minutes
Stems	2200 RPM	1	3 minutes
Roots	2500 RPM	1	3 minutes
Seeds	2800 RPM	1	3 minutes

NOTE: Homogenization should only be attempted within these guidelines. Exceeding these limits will stress the PowerPlant® Bead Tubes and may result in either tube breakage or leaking. Please call Technical Service at 1-800-606-6246 if you wish to explore the possibility of increasing the speed and homogenization time with the PowerLyzer™ 24.

NOTE: Please see the "Hints and Troubleshooting Guide" for other methods of homogenization. The PowerPlant® Bead Tubes are compatible with the Precellys® 24, Fastprep® machines, and all bead beater instruments. *However, the speed and length of homogenization time are critical to prevent tube breakage.*

7. Make sure the PowerPlant® Bead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature.
   CAUTION: Be sure not to exceed 13,000 x *g* or tubes may break.
8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect between 400 to 500 µl of supernatant. Supernatant may still contain some plant tissue particles.
9. Add 250 µl of Solution PB2 and invert the tubes to mix the contents. Incubate at 4°C for 5 minutes.
10. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g.*
11. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2.2 ml Collection Tube (provided).
12. Add 1 ml of Solution PB3 and invert the tubes at least 5 times to mix the contents. Incubate at room temperature for 10 minutes.
13. Centrifuge the tubes at room temperature for 15 minutes at 13,000 x *g.*
14. Discard the supernatant and resuspend the pellet in 100 µl of Solution PB6. Note: The tubes do NOT have to be air dried as residual isopropanol will not affect the process.
15. Shake to mix Solution PB4. Add 500 µl of Solution PB4 and vortex briefly to mix.
16. Load the entire volume (600 µl) onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute.
17. Remove the Spin Filter basket, discard the flow through, and replace the Spin Filter basket back in the tube.
18. Add 500 µl of Solution PB5 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.
19. Discard the flow through from the 2 ml Collection Tube.
20. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.
21. Carefully place Spin Filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution PB5 onto the Spin Filter.
22. Add 50 µl of Solution PB6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).
23. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
24. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20° to -80°C). Solution PB6 does not contain EDTA. To concentrate DNA see the Hints and Troubleshooting Guide.

NOTE: Occasionally, plants such as grapes, cotton, sunflower, strawberry, pine needles, etc. will yield DNA with inhibitors, which may prevent target sequences from amplifying in PCR. Under such circumstances, it is suggested to use the clean-up protocol provided. Alternatively you can dilute the template DNA one to several fold, for successful PCR.

Thank you for choosing the PowerPlant® DNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)
Please wear gloves at all times

This protocol is written for the first time user. It is designed to be informative and describes each step in detail. After understanding the principles involved in each step, it will be easier for the user to follow the Experienced User protocol.

1. To the PowerPlant® Bead Tubes provided, add up to 50 mg (0.05 g) of plant tissue sample, followed by the addition of 550 µl of PowerPlant® Bead Solution.
   NOTE: THE DNA EXTRACTION EFFICIENCY WILL IMPROVE BASED ON THE CONDITION OF HOMOGENIZATION AND THE TYPE OF HOMOGENIZATION METHOD. PLEASE REFER TO THE "ADDITIONAL INFORMATION" SECTION FOR DIFFICULT PLANT TYPES.

   What's happening: After your sample has been loaded into the PowerPlant® Bead Tube, the next step is a homogenization and lysis procedure. The PowerPlant® Bead Tube contains a buffer that will (a) help wet the tissue surfaces, and (b) protect nucleic acids from degradation.

2. Gently vortex to mix.

   What's happening: Gentle vortexing mixes the components in the PowerPlant® Bead Tube.

3. **Check Solution PB1**. If Solution PB1 has precipitated, heat solution to 60°C until the precipitate has dissolved before use.

   What's happening: Solution PB1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down proteins, fatty acids and lipids associated with the cell membranes. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. Solution PB1 can be used while it is still warm.

4. Add 60 µl of Solution PB1 and invert several times or vortex briefly.

5. Place the PowerPlant® Bead Tubes in a water bath at 65°C for 10 minutes.

   What's happening: Heating the plant tissues help in homogenizing them in the following step.

6. Homogenize using one of the following methods:

   A. Vortex:
      Secure PowerPlant® Bead Tubes horizontally using the MO BIO Vortex Adapter (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

   B. PowerLyzer™ Homogenizer:
      1. Properly identify each PowerPlant® Bead Tubes on both the cap and on the side.

   Note: Due to the high energies of the PowerLyzer™ 24, the potential of marring of the tops of the caps is possible, therefore it is recommended to mark the sides of the PowerPlant® Bead Tubes as well as the caps to ensure proper sample identification.

2. Place Bead Tubes into the Tube Holder of the PowerLyzer™ 24. The Bead Tubes must be balanced (evenly spaced) on the Tube Holder. Homogenize the tissue for 1 cycle at the chosen speed depending on your sample type for 3 minutes.

Plant Tissue Type	Speed	No. of Cycles	Time/Cycle
Soft leaf tissues	2000 RPM	1	3 minutes
Fibrous leaf tissues	2200 RPM	1	3 minutes
Stems	2200 RPM	1	3 minutes
Roots	2500 RPM	1	3 minutes
Seeds	2800 RPM	1	3 minutes

NOTE: Homogenization should only be attempted within these guidelines. Exceeding these limits will stress the PowerPlant® Bead Tubes and may result in either tube breakage or leaking. Please call Technical Service at 1-800-606-6246 if you wish to explore the possibility of increasing the speed and homogenization time with the PowerLyzer™ 24.

NOTE: Please see the "Hints and Troubleshooting Guide" for other methods of homogenization. The PowerPlant® Bead Tubes are compatible with the Precellys® 24, Fastprep® machines, and all bead beater instruments. *However, the speed and length of homogenization time are critical to prevent tube breakage.*

What's happening: The MO BIO Vortex Adapter is a simple platform to facilitate keeping the tubes tightly attached to the vortex. It is a highly recommended and cost effective way to obtain maximum DNA yields utilizing bead beating technology. Visit www.mobio.com for more information.

The PowerLyzer™ 24 Homogenizer is an instrument that provides highly effective lysing of biological samples. This unit provides high throughput and accommodates processing of up to 24 samples at once. Visit www.mobio.com/powerlyzer for more information.

7. Make sure the PowerPlant® Bead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature.
   CAUTION: Be sure not to exceed 13,000 x *g* or tubes may break.

8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

Note: *Expect between 400 to 500 µl of supernatant at this step. The exact recovered volume depends on the absorbency of your starting material and is not critical for the procedure to be effective. The supernatant may be dark green in appearance and still contain some tissue debris. The presence of carry over tissue debris or a dark color in the mixture is expected in many plant types at this step. Subsequent steps in the protocol will remove both carry over tissue debris and coloration of the mixture.*

9. Add 250 µl of Solution PB2 and invert the tubes to mix the contents. Incubate at 4°C for 5 minutes.

What's happening: Solution PB2 contains a reagent to precipitate non-DNA organic and inorganic material including plant polysaccharides, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

10. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.

11. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2.2 ml Collection Tube (provided).

What's happening: The pellet at this point contains non-DNA organic and inorganic material including plant tissue debris, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

12. Add 1 ml of Solution PB3 and invert the tubes at least 5 times to mix the contents. Incubate at room temperature for 10 minutes.

What's happening: Solution PB3 is 99% isopropanol and will precipitate DNA along with some organic contaminants. Most of the co-extracted impurities will be removed at this step.

13. Centrifuge the tubes at room temperature for 15 minutes at 13,000 x *g.*

14. Discard the supernatant and resuspend the pellet in 100 µl of Solution PB6. Note: The tubes do NOT have to be air dried as residual isopropanol will not affect the process.

What's happening: Isopropanol will precipitate and pellet the DNA. The pellet at this point contains relatively pure DNA along with some organic contaminants; mostly polysaccharides and phenolics depending on the plant tissues processed, leaving a majority of the contaminants in solution.

15. Shake to mix Solution PB4. Add 500 µl of Solution PB4 and vortex briefly to mix.

What's happening: Solution PB4 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filters.

16. Load the entire volume (600 µl) onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute.

What's happening: DNA is selectively bound to the silica membrane in the Spin Filter device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

17. Remove the Spin Filter basket, discard the flow through and replace the Spin Filter basket back in the tube.

18. Add 500 µl of Solution PB5 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.

What's happening: Solution PB5 is an ethanol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salts and other contaminants while allowing the DNA to stay bound to the silica membrane.

19. Discard the flow through from the 2 ml Collection Tube.

What's happening: This flow through fraction is non-DNA organic and inorganic waste removed from the silica Spin Filter membrane by the ethanol wash solution.

20. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.

What's happening: This second spin removes residual Solution PB5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution PB5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

21. Carefully place Spin Filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution PB5 onto the Spin Filter.

Note: *It is important to avoid any traces of the ethanol based wash solution.*

22. Add 50 µl of Solution PB6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).

    Note: *Placing the Solution PB6 in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution PB6 (elution buffer) passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution PB6 (10 mM Tris) which is a low salt solution.*

    Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution from the silica Spin Filter membrane at this step (MO BIO Catalog#. 17000-10). Solution PB6 contains no EDTA. If DNA degradation is a concern, Sterile TE may also be used instead of Solution PB6 for elution of DNA from the Spin Filter.

23. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.

24. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20° to -80°C). Solution PB6 does not contain any EDTA. To concentrate DNA see the Hints and Troubleshooting Guide.

NOTE: Occasionally, plants such as grapes, cotton, sunflower, strawberry, pine needles, etc. will yield DNA with inhibitors, which may prevent target sequences from amplifying in PCR. Under such circumstances, it is suggested to use the clean-up protocol provided. Alternatively you can dilute the template DNA one to several fold, for successful PCR.

Thank you for choosing the PowerPlant® DNA Isolation Kit.

Clean-Up Protocol

Use this protocol to improve the functional quality of extracted DNA if it does not successfully amplify in PCR. This clean-up step uses our patented Inhibitor Removal Technology® (IRT).

NOTE: This protocol requires additional 1.5 - 2.0 ml microcentrifuge tubes which are user provided.

1. Bring the volume of the eluted DNA up to 150 µl using sterile water and add 70 µl of PowerPlant® Bead Solution. Gently invert 3-5 times to mix.
2. Check Solution PB1. If it has precipitated, heat solution to 60°C until dissolved before use.
3. Add 20 µl of Solution PB1 and invert 3-5 times to mix.
4. Add 85 µl of Solution PB2 and invert 3-5 times to mix. Incubate at 4°C for 5 minutes.
5. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.
6. Avoiding the pellet, transfer the entire volume to another microcentrifuge tube, add 70 µl of Solution PB7 and invert 3-5 times to mix. Incubate at 4°C for 5 minutes.
7. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*.
8. Avoiding the pellet, transfer the supernatant to another microcentrifuge tube. Add 0.5 ml of Solution PB3 and invert 3-5 times to mix.
9. Incubate at room temperature for 10 minutes followed by centrifugation at 13,000 x *g* for 10 minutes.
10. Decant the supernatant and air dry the tubes until they are free of residual isopropanol.
11. Resuspend the DNA in Solution PB6. Use appropriate volumes to reconstitute the pellet; usually the original starting volume of DNA is used. A reduced volume may be used to obtain a more concentrated solution of DNA. Sterile water can also be used to reconstitute the DNA instead of Solution PB6.

Hints and Troubleshooting Guide

Plant Types

Plant tissues vary widely in their composition but predominately consist of polysaccharides and polyphenols. The efficiency of DNA isolation is dictated by these two components because polyphenols and polysaccharides can complex with nucleic acid molecules and initiate their degradation very rapidly thus preventing their isolation for further downstream applications. This detrimental association can be avoided by grinding the samples in the absence of any liquid. The PowerPlant® DNA Isolation Kit offers the advantage of either grinding the samples dry, in the absence of any liquid or it can be done in the presence of the PowerPlant® Bead Solution as per the protocol. We recommend homogenizing the plant samples such as grapes, strawberry, etc. dry, in the absence of any liquid. After homogenizing the samples, you can add the PowerPlant® Bead Solution and proceed with the protocol.

Methods of Homogenization

Plant tissues are usually tough and fibrous which makes lysing the cells difficult; often requiring mechanical homogenization, hand grinding, or freezing and grinding in liquid nitrogen. A vast majority of leaf tissues are soft and can be processed for DNA isolation with MO BIO's PowerPlant® DNA Isolation Kit by means of a simple vortex adapter or hand vortexing for a few minutes. However other plant tissues such as roots, wood tissues, and plant seeds are tough to homogenize and thus require the use of mechanical homogenization. The versatile nature of MO BIO's PowerPlant® DNA Isolation Kit chemistry makes it compatible with most other methods of homogenization. The following methods have been validated with this kit:

- **Homogenization by hand**
  This is achieved through simple grinding with a micro pestle in a microcentrifuge tube. Up to 100 mg of plant tissue material has been consistently used and homogenized sufficiently with this method. Please note that it is strenuous to handle a large of number of samples using this method. Another option is to use a pestle and mortar which is much easier. However, this is effective only when a large amount of plant material is processed. A known amount of material (usually 50 mg) can then transferred to the PowerPlant® Bead Tubes and processed as per the protocol.

- **Homogenization with PowerLyzer™ 24**
  Up to 24 samples can be homogenized in 2 ml screw cap tubes. This method is fast because the homogenization time is reduced and 24 samples can be processed at one time. The following table serves as a guide to determine the optimal speed, number of cycles, and homogenization cycle time.
  NOTE: Homogenization should only be attempted within these guidelines. Exceeding these limits will stress the PowerPlant® Bead Tubes and may result in either tube breakage or leaking. Please call Technical Service at 1-800-606-6246 if you wish to explore the possibility of increasing the speed and homogenization time with the PowerLyzer™ 24.

- **Homogenization with Retsch Shaker**
  A tube adapter (MO BIO Catalog# 11999) is needed in order to process the PowerPlant® Bead Tubes in the Retsch Shaker. The recommended speed for this machine is 20 for two cycles of 5 minutes each. The samples are placed in the tube adaptor and homogenized for 5 minutes and then the tube adaptor is turned around vertically and the samples are homogenized for another 5 minutes. For fibrous tissues and seeds the recommended time is 20 minutes, 10 minutes on each side of the block, for effective homogenization.

Hints and Troubleshooting Guide cont.

- **Other Methods**
  For the use of other homogenizers such as the FastPrep®, BioSpec, or Genogrinder, please call Technical Service at 800-606-6246. The PowerPlant® Bead Tubes are high density tubes, however, it is safer to consult with our Technical Service Department before deciding the speed and time of homogenization with other homogenizers.

- **Liquid Nitrogen**
  Liquid nitrogen is another powerful method of homogenization; however there are safety issues involved. If using liquid nitrogen, transfer the plant material into a microcentrifuge that is rated for liquid nitrogen use (call technical services if you are not sure) and freeze in liquid nitrogen for up to 10 minutes. Then, using a sterile micro pestle, grind the tissues to a paste as fast as possible. Resuspend the paste in the PowerPlant® Bead Solution and transfer it to a PowerPlant® Bead Tube and proceed with the protocol.

Concentrating the DNA

The final volume of eluted DNA will be 50 µl. The DNA may be concentrated by adding 5 µl (1/10th volume) of 5M NaCl and inverting 3-5 times to mix. Next, add 100 µl of 100% cold ethanol and invert 3-5 times to mix. Incubate at -20°C for up to 30 minutes and centrifuge at 10,000 x *g* for 15 minutes at room temperature. Decant all liquid. (If sterile DNA is desired, wash the DNA pellet with 70% cold ethanol. Be sure not to disturb the pellet.) Remove residual ethanol in a speed vac, dessicator, or ambient air. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.

If DNA Does Not PCR Amplify

- Check DNA yield by gel electrophoresis and spectrophotometer reading. Template is typically added to 10 ng per reaction, although more or less may be needed depending on the reaction conditions, enzyme activity, and copy number of the target sequence.
- If DNA does not amplify after altering the amount of template in the reaction, PCR optimization (i.e. changing reaction conditions, validating primers, or testing a different polymerase) may be needed.

DNA Floats Out of Well When Loaded on a Gel

This usually occurs because residual Solution PB5 remains in the final sample. Prevent this by being careful not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation (described in "Concentrating the DNA") is the best way to remove residual Solution PB5.

Storing DNA

DNA is eluted in Solution PB6 (10mM Tris). Store the DNA at -20°C to prevent degradation. DNA can be eluted in TE without DNA loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA that has been eluted into sterile water should be stored at -70°C.

Contact Information

Technical Support:
Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: technical@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

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For the distributor nearest you, visit our website at www.mobio.com/distributors

Other Quality Products Available from MO BIO Laboratories, Inc.

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DNA Purification and Gel Extraction	Catalog No.	Quantity
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
UltraClean® 15 DNA Purification Kit	12100-300	300 preps
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps
UltraClean®-htp 96 Well PCR Clean-Up Kit	12596-4 12596-12	4 x 96 preps 12 x 96 preps
UltraClean® GelSpin® DNA Extraction Kit	12400-50 12400-100 12400-250	50 preps 100 preps 250 preps
Plasmid DNA Isolation	**Catalog No.**	**Quantity**
UltraClean® 6 Minute Mini Plasmid Prep Kit	12300-50 12300-100 12300-250	50 preps 100 preps 250 preps
UltraClean® Standard Mini Plasmid Prep Kit	12301-50 12301-100 12301-250	50 preps 100 preps 250 preps
UltraClean®-htp 96 Well Plasmid Prep Kit	12396-4 12396-12	4 x 96 preps 12 x 96 preps
UltraClean® Midi Plasmid Prep Kit	12700-20 12700-50	20 preps 50 preps
UltraClean® Maxi Plasmid Prep Kit	12600-10 12600-20	10 preps 20 preps
UltraClean® Endotoxin-Free Mini Plasmid Prep Kit	12311-100 12311-250	100 preps 250 preps
UltraClean® Endotoxin-Free Midi Plasmid Prep Kit	12711-10	10 preps
UltraClean® Endotoxin-Free Maxi Plasmid Prep Kit	12611-10	10 preps
UltraClean® Endotoxin Removal Kit	12615	1 kit
UltraClean® Endotoxin-Free Ethanol Precipitation Kit	12616	1 kit
UltraClean® Endotoxin Removal Reagent	12625-25	25 ml
Endotoxin-Free Sodium Chloride	12626-15	15 ml
Endotoxin-Free Centrifuge Tubes	12617-100 12618-50 12619-25	100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes
RNA Isolation	**Catalog No.**	**Quantity**
PowerLyzer™ UltraClean® Tissue & Cells RNA Isolation Kit	15055-50	50 preps
PowerLyzer™ UltraClean® Plant RNA Isolation Kit	13355-50	50 preps
PowerBiofilm™ RNA Isolation Kit	25000-50	50 preps
LifeGuard™ Soil Stabilization Solution	12868-10 12868-100 12868-1000 12868-7500	10 ml 100 ml 1 L 7.5 L
On-Spin Column DNase I Kit (RNase-Free)	15100-50	50 preps
BiOstic® Stabilized Blood RNA Isolation Kit	12231-20 12231-50 12231-100	20 preps 50 preps 100 preps
BiOstic® Blood Total RNA Isolation Kit	12230-20 12230-50	20 preps 50 preps

RNA Isolation …*Continued*	Catalog No.	Quantity
RNA PowerSoil® DNA Elution Accessory Kit	12867-25	25 preps
RNA PowerSoil® Total RNA Isolation Kit	12866-25	25 preps
UltraClean® Microbial RNA Isolation Kit	15800-50 15800-250	50 preps 250 preps
UltraClean® Tissue & Cells RNA Isolation Kit	15000-50 15000-250	50 preps 250 preps
UltraClean® Plant RNA Isolation Kit	13300-20 13300-50	20 preps 50 preps
Genomic DNA Isolation	**Catalog No.**	**Quantity**
PowerLyzer™ PowerSoil® DNA Isolation Kit	12855-50	50 preps
PowerLyzer™ UltraClean® Microbial DNA Isolation Kit	12255-50	50 preps
PowerBiofilm™ DNA Isolation Kit	24000-50	50 preps
PowerFood™ Microbial DNA Isolation Kit	21000-50 21000-100	50 preps 100 preps
BiOstic® Bacteremia DNA Isolation Kit	12240-50	50 preps
BiOstic® FFPE Tissue DNA Isolation Kit	12250-50	50 preps
BiOstic® Paraffin Removal Reagent	12251-50	2 x 25 ml
PowerMax® Soil DNA Isolation Kit	12988-10	10 preps
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
PowerSoil®-htp 96 Well Soil DNA Isolation Kit	12955-4 12955-12	4 x 96 preps 12 x 96 preps
UltraClean® Soil DNA Isolation Kit	12800-50 12800-100	50 preps 100 preps
UltraClean®-htp 96 Well Soil DNA Isolation Kit	12896-4 12896-12	4 x 96 preps 12 x 96 preps
UltraClean® Mega Soil DNA Isolation Kit	12900-10	10 preps
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
UltraClean® Fecal DNA Isolation Kit	12811-50 12811-100	50 preps 100 preps
PowerMicrobial® Midi DNA Isolation Kit	12225-25	25 preps
PowerMicrobial® Maxi DNA Isolation Kit	12226-25	25 preps
UltraClean® Microbial DNA Isolation Kit	12224-50 12224-250	50 preps 250 preps
UltraClean®-htp 96 Well Microbial DNA Isolation Kit	10196-4 10196-12	4 x 96 preps 12 x 96 preps
PowerPlant® DNA Isolation Kit	13200-50 13200-100	50 preps 100 preps
UltraClean® Plant DNA Isolation Kit	13000-50 13000-250	50 preps 250 preps

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Genomic DNA Isolation *...Continued*	Catalog No.	Quantity
UltraClean®-htp 96 Well Plant DNA Isolation Kit	13096-4 13096-12	4 x 96 preps 12 x 96 preps
UltraClean® Tissue & Cells DNA Isolation Kit	12334-50 12334-250	50 preps 250 preps
UltraClean®-htp 96 Well Tissue DNA Isolation Kit	12996-4 12996-12	4 x 96 preps 12 x 96 preps
UltraClean® Blood DNA Isolation Kit (Non-Spin)	12000-100	100 preps
UltraClean® Blood DNA Isolation Kit (Processes 1,000 ml of Blood)	12000-1000	1 kit
UltraClean® Blood DNA Isolation Kit Plus RNase (Processes 1,000 ml of Blood)	12002-1000	1 kit
UltraClean® BloodSpin® DNA Isolation Kit	12200-50 12200-250	50 preps 250 preps
UltraClean®-htp 96 Well BloodSpin® DNA Isolation Kit	12296-4 12296-12	4 x 96 preps 12 x 96 preps
UltraClean® Forensic DNA Isolation Kit	14000-10 14000-20	10 isolations 20 isolations
PowerWater® DNA Isolation Kit	 14900-50-NF 14900-50-22 14900-50-45 14900-100-NF 14900-100-22 14900-100-45	50 preps (No filters) (0.22 µm) (0.45 µm) 100 preps (No filters) (0.22 µm) (0.45 µm)
RapidWater™ DNA Isolation Kit	 14810-50-NF 14810-50-22 14810-50-45 14810-100-NF 14810-100-22 14810-100-45	50 preps (No filters) (0.22 µm) (0.45 µm) 100 preps (No filters) (0.22 µm) (0.45 µm)
UltraClean® Water DNA Isolation Kit (0.45µm filters)	14800-10 14800-25	10 preps 25 preps
UltraClean® Water DNA Isolation Kit (0.22 µm filters)	14880-10 14880-25	10 preps 25 preps
UltraClean® Water DNA Isolation Kit (No filters)	14800-10-NF 14800-25-NF	10 preps 25 preps

Microbiological Culture Media	Catalog No.	Quantity
TB DRY® Powder Growth Media	12105-05 12105-1 12105-5	500 g 1 kg 5 kg
LB Broth Powder Growth Media, pH 7	12106-05 12106-1 12106-5	500 g 1 kg 5 kg
LB Agar Powder Growth Media, pH 7	12107-05 12107-1 12107-5	500 g 1 kg 5 kg
LB Broth (Lennox) Powder Growth Media, pH 7	12108-05 12108-1 12108-5	500 g 1 kg 5 kg

Other Reagents and Lab Accessories	Catalog No.	Quantity
LB Agar (Lennox) Powder Growth Media, pH 7	12109-05 12109-1 12109-5	500 g 1 kg 5 kg
Soybean-Casein Digest Medium (TSB), USP	12114-05 12114-1 12114-5	500 g 1 kg 5 kg
Soybean-Casein Digest Agar Medium (TSA), USP	12115-05 12115-1 12115-5	500 g 1 kg 5 kg
Yeast Extract	12110-05 12110-1 12110-5	500 g 1 kg 5 kg
Tryptone	12111-05 12111-1 12111-5	500 g 1 kg 5 kg
Agar, Bacteriological Grade	12112-05 12112-1 12112-5	500 g 1 kg 5 kg
20 bp DNA Ladder	17020-40	40 µg
100 bp DNA Ladder	17100-40	40 µg
1 kb DNA Ladder	17200-100	100 µg
UltraClean® Agarose, Molecular Biology Grade	15003-50 15003-100 15003-500 15003-1000	50 g 100 g 500 g 1 kg
UltraClean® MS-8 Agarose	15515-50 15515-100 15515-500	50 g 100 g 500 g
UltraClean® Forensic Agarose	15505-50 15505-100 15505-500	50 g 100 g 500 g
UltraClean® Low Melt Agarose	15005-50 15005-100 15005-500	50 g 100 g 500 g
UltraClean® Low Melt Sieve Agarose	15004-50 15004-100 15004-500	50 g 100 g 500 g
Ethidium Bromide Solution	15006-1 15006-10	1 ml 10 ml
Ethidium Bromide Destaining Tea Bags	15007-25	25 bags
Bromophenol Blue Gel Loading Buffer	15008-1 15008-5	1 ml 5 x 1 ml
Bromophenol Blue/Xylene Cyanol Gel Loading Buffer	15009-1 15009-5	1 ml 5 x 1 ml
TAE Buffer, 50X (Tris-acetate-EDTA)	15001-100 15001-500 15001-1000	100 ml 500 ml 1 liter

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Other Reagents and Lab Accessories…*Continued*	Catalog No.	Quantity
TBE Buffer, 10X (Tris-borate-EDTA)	15002-100 15002-500 15002-1000	100 ml 500 ml 1 liter
RNase-Free Gloves	1555-XS 1555-S 1555-M 1555-L	bag of 100 bag of 100 bag of 100 bag of 100
UltraClean® Lab Cleaner	12095-250 12095-500 12095-1000	250 ml squeeze bottle 500 ml spray bottle 1 liter bottle
KAPA PROBE FAST qPCR Kits	51220-100 51220-500 51220-1000	100 reactions 500 reactions 1000 reactions
KAPA SYBR® FAST Universal 2X qPCR Master Mix	51230-100 51230-500 51230-1000	100 reactions 500 reactions 1000 reactions
KAPA2G Robust HotStart ReadyMix	51240-100 51240-500	100 reactions 500 reactions
KAPA HiFi HotStart ReadyMix	51250-100 51250-500	100 reactions 500 reactions
KAPA2G FAST HotStart DNA Polymerase with dNTPs	51260-100 51260-250 51260-500	100 reactions 250 reactions 500 reactions
KAPA2G FAST HotStart ReadyMix	51270-100 51270-500	100 reactions 500 reactions
KAPA Long Range HotStart DNA Polymerase with dNTPs	51280-100 51280-250 51280-500	100 reactions 250 reactions 500 reactions
KAPA Taq Polymerase ReadyMix	51290-250	250 reactions
OmniTaq™ DNA Polymerase Enzyme	1224-250	250 reactions (10 U/µl)
OmniTaq™ DNA Polymerase 2x Master Mix	1226-250	250 reactions (5 x 1.25 ml/tube)
Omni KlenTaq™ DNA Polymerase Enzyme	1225-250	250 reactions (25 U/µl)
Omni KlenTaq™ DNA Polymerase 2x Master Mix	1227-250	250 reactions (5 x 1.25 ml/tube)
DNase (RNase-Free)	15600-5 15601-100	5 mg 2500 units
Proteinase K	1223-100 1222-2	100 mg 2 ml (20 mg/ml)
Ribonuclease A (25 mg/ml)	1202-1 1202-5	1 ml 5 ml
PCR Water	17000-1 17000-5 17000-10 17000-11	1 ml 5 x 1 ml 10 x 1 ml 10 ml bottle
Molecular Biology Grade Water	17012-200 17012-5200	200 ml 5 x 200 ml
DEPC Treated Water	17011-200 17011-5200	200 ml 5 x 200 ml
Endotoxin-Free Water	17013-10 17013-50 17013-100 17013-500	10 ml 50 ml 100 ml 500 ml

Instrumentation and Accessories	Catalog No.	Quantity
PowerLyzer™ 24 Bench Top Bead-Based Homogenizer (110/220V)	13155	1 unit
PowerLyzer™ Tube Holder	13156	1 unit
PowerLyzer™ Tube Holder Stand	13157	1 unit
PowerVac™ Mini System	11992	1 unit + 20 adapters
PowerVac™ Manifold	11991	1 unit
PowerVac™ Mini Spin Filter Adapters	11992-10 11992-20	10 adapters 20 adapters
Ceramic Bead Tubes, 1.4 mm	13113-50	50 bead tubes
Ceramic Bead Tubes, 2.8 mm	13114-50	50 bead tubes
Glass Bead Tubes, 0.5 mm	13116-50	50 bead tubes
Glass Bead Tubes, 0.1 mm	13118-50	50 bead tubes
Metal Bead Tubes, 2.38 mm	13117-50	50 bead tubes
2.0 ml Tough Tubes with Cap	13119-500 13119-1000	500 1000
Carbide Bead Tubes, 0.25 mm	13121-50	50 x 0.5 ml tubes
Garnet Bead Tubes, 0.15 mm	13122-50	50 x 0.5 ml tubes
Garnet Bead Tubes, 0.70 mm	13123-50	50 x 2 ml tubes
Garnet + ¼ Ceramic 15 ml Bead Tubes, 0.70 mm	13134-50	50 tubes
Garnet + ¼ Ceramic 50 ml Bead Tubes, 0.70 mm `	13144-10 13144-50 13144-100 13144-500	10 tubes 50 tubes 100 tubes 500 tubes
Glass 15 ml Bead Tubes, 0.1 mm	13135-50	50 tubes
Glass 50 ml Bead Tubes, 0.1 mm	13145-10 13145-50 13145-100 13145-500	10 tubes 50 tubes 100 tubes 500 tubes
Glass 15 ml Bead Tubes, 1.0 mm	13136-50	50 tubes
Ceramic 15 ml Bead Tubes, 1.4 mm	13137-50	50 tubes
Ceramic 50 ml Bead Tubes, 1.4 mm	13147-10 13147-50	10 tubes 50 tubes
Metal 50 ml Bead Tubes, 2.38 mm	13149-10 13149-50	10 tubes 50 tubes

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Instrumentation and Accessories… *Continued*	Catalog No.	Quantity
PowerMix 15 ml Bead Tubes	13138-50	50 tubes
PowerMix 50 ml Bead Tubes	13148-10 13148-50	10 tubes 50 tubes
2 ml Collection Tubes	1200-100-T 1200-150-T 1200-250-T	100 tubes 150 tubes 250 tubes
2 ml Screw Cap Tubes	12800-200-E	200 tubes & caps
15 ml Collection Tubes	12700-T	25 tubes
50 ml Centrifuge Tubes	12600-T	25 tubes
Spin Filters (in 1.9 ml tubes)	1200-50-SF 1200-100-SF 1200-250-SF	50 filters 100 filters 250 filters
Endotoxin-Free Centrifuge Tubes	12617-100 12618-50 12619-25	100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes
15 ml Midi Spin Filters	12700-SF	25 spin filters
Vortex-Genie® 2 Vortex (120V)	13111-V	1 unit
Vortex-Genie® 2 Vortex (220V)	13111-V-220	1 unit
Vortex Adapter, holds 12 (1.5-2.0 ml) tubes	13000-V1	1 unit
Vortex Adapter, holds 6 (5 ml) tubes	13000-V1-5	1 unit
Vortex Adapter, holds 4 (15 ml) tubes	13000-V1-15	1 unit
Vortex Adapter, holds 2 (50 ml) tubes	13000-V1-50	1 unit
Vortex Adapter, holds 24 (1.5-2.0 ml) tubes	13000-V1-24	1 unit
BagMixer® 400 VW	23112	1 unit
BagFilter® 400 P	23113-500	Box of 500
BagPage® 400	23114-500	Box of 500

Instrumentation and Accessories… *Continued*	Catalog No.	Quantity
Whirl-Pak® Collection Bag, Medium (1,627 ml)	23211-500	500 bags
Whirl-Pak® Collection Bag, Large (3,637 ml)	23212-250	250 bags
Whirl-Pak® Stand up Bag, Small (118 ml)	23220-500	500 bags
Whirl-Pak® Stand up Bag, Medium (532 ml)	23221-500	500 bags
Whirl-Pak® Stand up Bag, Large (1,242 ml)	23222-250	250 bags
Whirl-Pak® Stand up Bag, Extra-Large (2,041 ml)	23223-250	250 bags
Whirl-Pak® Scoop Bag, 60 ml	23240-50	50 bags
Anti-Static Funnels, Micro	23301-96	Pack of 96
Anti-Static Funnels, Small	23302-50	Pack of 50
Anti-Static Funnels, Medium	23303-50	Pack of 50
Anti-Static Funnels, Large	23304-20	Pack of 20
Mini Horizontal Gel System	16001	1 each
Mini Horizontal Gel Caster, 3 place	16003	1 each
Mini Horizontal Gel Tray	16004	1 each
Polycarbonate Single-sided Comb	16005 16006 16007 16008	1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well
Polycarbonate Dual-sided Comb	16013 16014 16015 16016	1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well
Teflon Single-sided Comb	16009 16010 16011 16012	1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well
Teflon Dual-sided Comb	16017 16018 16019 16020	1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well
Power Supply w/Timer, (120V)	16023	1 unit

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Instrumentation and Accessories… *Continued*	Catalog No.	Quantity
96 Well Plate Shaker (120V)	11996	1 unit
96 Well Plate Shaker (220V)	11996-220	1 unit
Plate Adapter Set	11999	1 set

Instrumentation and Accessories… *Continued*	Catalog No.	Quantity
Vacuum Pump (120V)	11998	1 unit
Vacuum Pump (220V)	11998-220	1 unit