PowerPlant® Pro DNA Isolation Kit

Catalog No.	Quantity
13400-50	50 Preps

Instruction Manual

Inhibitor Removal Technology® (IRT) is a registered trademark of MO BIO Laboratories, Inc. and is covered by the following patents USA US 7,459,548 B2, Australia 2005323451, Japan 5112064 and India 246946.

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Version: 08162013

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Introduction

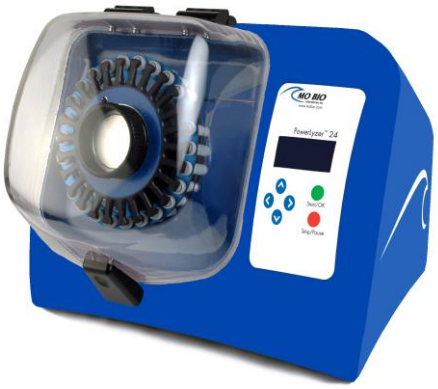
The PowerPlant® Pro DNA Isolation Kit is designed for fast and easy purification of total cellular DNA from plant cells, tissues and seeds. The bead beating technology used in this kit replaces cumbersome DNA isolation procedures such as CTAB, phenol, or chloroform extraction for recovery of high quality DNA from the toughest sample types, including strawberry leaf, cotton leaf, cotton seeds, and pine needles. The PowerPlant® Pro DNA Isolation Kit utilizes our patented Inhibitor Removal Technology® (IRT) for removal of PCR inhibitors from plant extracts during the isolation process, resulting in DNA that is ready to use in any downstream applications including PCR, qPCR and sequencing.

Protocol Overview

Plant samples from 5 - 50 mg are added to a bead tube along with a kit supplied buffer for rapid homogenization. Cell lysis and DNA release occurs by mechanical and chemical methods. Released genomic DNA is cleared of PCR inhibitors using IRT and then DNA is 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.

Mechanical Lysis Options

The PowerPlant® Pro DNA Isolation Kit may be used with a vortex or high velocity bead beater, such as the PowerLyzer™ 24 homogenizer. The PowerLyzer™ 24 is suitable for fast homogenization of plant materials including stems, roots, seeds or difficult leaf tissue without the need of liquid nitrogen grinding.



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

Using the PowerPlant® Pro DNA Isolation Kit with the PowerLyzer™24 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™ 24, guidelines for getting started can be found in step 4 on pages 8 and 10 of the protocol.

Using the PowerPlant® Pro 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.

Phenolic Separation Solution (PSS)

For plant samples high in polyphenolic compounds, the addition of the Phenolic Separation Solution is recommended. The Phenolic Separation Solution prevents loss of nucleic acids by preventing irreversible binding to phenolics which are removed by IRT. Not all plant samples require the addition of PSS. Examples of plant tissues that require the addition of PSS are pine and grape leaf. If you are unsure of the phenolic content of your samples, evaluate the DNA recovery with and without PSS to determine the optimal protocol.

Average DNA Yields

DNA yields from plant tissues can vary based on the age and type of tissue and the level of phenolic compounds. Below are estimated DNA yields from a variety of plant samples evaluated using the PowerPlant® Pro DNA Isolation Kit.

Plant Sample	DNA yields (50mg)	PSS
Grape leaf	2.5-3.5 µg	+
Strawberry leaf	10-15 µg	+
Tomato stem	10-25 µg	+
Cotton leaf	2.5-3.5 µg	+/-
Cotton seed	20-25 µg	-
Grass leaf	40-50 µg	-
Pine needle	30-35 µg	+
Rice leaf	7-11 µg	-
Mint leaf	2-3 µg	-

+, yields were improved with the addition of PSS, **-,** yields were reduced with the addition of PSS, **+/-,** yields were the same with or without PSS.

High Throughput Options

For additional high throughput options MO BIO offers the PowerPlant® Pro-htp 96 Well 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 & 11990, respectively.) Please contact Technical Support with questions on 96 well DNA isolation.

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

Other Related Products	Catalog No.	Quantity
PowerPlant® Pro-htp 96 Well DNA Isolation Kit	13496-4	4 x 96 preps
PowerPlant® RNA Isolation Kit	13500-50	50 preps
PowerPlant® RNA Isolation Kit with DNase	13550-50	50 preps
PowerLyzer™ 24 Homogenizer	13155	1 unit
Vortex Adapter, holds 24 (1.5-2.0 ml) tubes	13000-V1-24	1 unit

PowerPlant® Pro DNA Isolation Kit

Prepare Sample

- Add up to 50 mg of plant tissue to the PowerPlant® Bead Tube
- Add Solution PD1

**Optional: Add Phenolic Separation Sol'n*

Cell Lysis

- Add Solution PD2 and RNase A
- Attach to Vortex Adapter or place into the PowerLyzer™ 24
- Homogenize

Centrifuge

Inhibitor Removal Technology®

- Add Solution PD3
- Incubate at 4°C

Centrifuge

Bind DNA

- Add Solution PD4/PD6
- Load into Spin Filter

Centrifuge

Wash

- Wash with Solution PD5
- Wash with Solution PD6

Centrifuge

Elute

- Elute with Solution PD7

Equipment Required

Microcentrifuge (up to 16,000 x *g*)
Pipettor (volumes required 50 – 600 µl)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220), PowerLyzer™ 24 Homogenizer or similar instrument

Kit Contents

	Kit Catalog # 13400-50	
Component	**Catalog#**	**Amount**
Solution PD1	13400-50-1	25 ml
Solution PD2	13400-50-2	3 ml
Solution PD3	13400-50-3	14 ml
Solution PD4	13400-50-4	32 ml
Solution PD5	13400-50-5	28 ml
Solution PD6	13400-50-6	2 x 30 ml
Solution PD7	13400-50-7	5.5 ml
RNase A Solution (25 mg/ml)	13400-50-8	165 µl
Phenolic Separation Solution	13400-50-9	2.2 ml
PowerPlant® Bead Tubes	13400-50-BT	50
Spin Filters	13400-50-SF	50
2 ml Collection Tubes	13400-50-T	150

Kit Storage

RNase A should be stored at 4°C.
The other 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: Solutions PD5 & PD6 contain ethanol. They are flammable.

IMPORTANT NOTES FOR USE: Check **Solution PD2** for precipitates. If the solution contains precipitates, heat at 37°C-55°C to dissolve.

Experienced User Protocol

Please wear gloves at all times

1. To the 2 ml **PowerPlant® Bead Tubes** provided, add up to 50 mg of fresh plant tissue and 450 µl of **Solution PD1**.
   Note: It is recommended that the tissue be cut into small pieces prior to loading it into the bead tube. For tough plants or seeds pre-grind the material with a mortar and pestle**.**
   Note: If your sample is high in phenolics (see page 5) and you are using the **Phenolic Separation Solution**, reduce **Solution PD1 to 410 µl and add 40 µl of the Phenolic Separation Solution.**

2. Check **Solution PD2** for precipitates, if precipitated, warm at 37°C - 55°C until dissolved. Add 50 µl of **Solution PD2**.

3. Add 3 µl of **RNase A Solution** to the PowerPlant® Bead Tube and vortex briefly to mix.

4. Homogenize using one of the following methods:
   Note: See ***Heating of Samples Prior to Bead Beating*** in the **Hints and Troubleshooting Guide (page 13)**.

   A. Vortex:
   Secure PowerPlant® Bead Tubes horizontally to the MO BIO Vortex Adapter (MO BIO Catalog# 13000-V1-24) or on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
   Note: Most leaf tissues are soft and can be processed for DNA isolation by using a vortex adapter. However, plant tissues such as roots, wood, and plant seeds require pre-grinding with a mortar and pestle before placing on the vortex.

   B. PowerLyzer™ 24 Homogenizer:
      1. Properly identify each PowerPlant® Bead Tube on both the cap and on the side.

         Note: Due to the high energies of the PowerLyzer™ 24, the potential for marring of the cap tops 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 PowerPlant® 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 2 minutes.

Plant Tissue Type	Speed	No. of Cycles	Time/Cycle
Soft leaf tissues	2000 RPM	1	2 minutes
Fibrous leaf tissues	2200 RPM	1	2 minutes
Stems	2200 RPM	1	2 minutes
Roots	2500 RPM	1	2 minutes
Pine needles	2600 RPM	1	2 minutes
Seeds	2800 RPM	1	2 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 Support at 1-800-606-6246 if you wish to explore the possibility of increasing the speed and homogenization time with the PowerLyzer™ 24.

5. Centrifuge Bead Tubes at 13,000 x *g* for 2 minutes.

6. Transfer the supernatant to a clean **2 ml Collection Tube** (provided).
   Note: With 50 mg of plant tissue and depending upon plant type, expect 450 – 550 µl of supernatant, which may contain some particles.

7. Add 175 µl of **Solution PD3**. Vortex 5 seconds. Incubate at 4°C for 5 minutes.
   Note: For problematic samples you can add up to 250 µl of PD3 at this step. It is best to start at 175 µl for most sample types.

8. Centrifuge the Collection Tube for 2 minutes at 13,000 x *g.*

9. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean **2 ml Collection Tube** (provided).

10. Add 600 µl of **Solution PD4** and 600 µl of **Solution PD6**. Vortex to mix for 5 seconds.

11. Load approximately 600 µl of lysate onto the **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds. Discard the flow through, place the Spin Filter back into the Collection Tube and add another 600 µl of lysate and centrifuge at 10,000 x *g* for 30 seconds. Discard the flow-through and repeat a third time until all of the lysate has been passed through the Spin Filter. Discard the flow-through and place the Spin Filter back into the Collection Tube.

12. Add 500 µl of **Solution PD5** to the Spin Filter column. Centrifuge for 30 seconds at 10,000 x *g*. Discard the flow through. Place the Spin Filter back into the same Collection Tube.

13. Add 500 µl of **Solution PD6** to the Spin Filter column. Centrifuge for 30 seconds at 10,000 x *g*. Discard the flow through. Place the Spin Filter back into the same Collection Tube.

14. Centrifuge again for 2 minutes at up to 16,000 *x g* to remove residual **Solution PD6**.

15. Carefully place the **Spin Filter** into a new clean **2 ml Collection Tube** (provided). Avoid splashing any **Solution PD6** onto the **Spin Filter**.

16. Add 50 - 100 µl of **Solution PD7** (10 mM Tris, pH 8.0) to the center of the white filter membrane and incubate for 2 minutes at room temperature.

17. Centrifuge 30 seconds at 10,000 *x g*.
    Note: For maximum elution efficiency re-load the flow through once again to the center of the white filter membrane. Centrifuge 30 seconds at 10,000 *x g*.

18. Discard the **Spin Filter**. DNA in the tube is now ready to use. No further steps are required.

We recommend storing DNA frozen (-20°C). **Solution PD7** contains no EDTA.

Thank you for choosing the PowerPlant® Pro DNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)

Please wear gloves at all times

1. To the 2 ml **PowerPlant® Bead Tubes** provided, add up to 50 mg of fresh plant tissue and 450 µl of **Solution PD1**.
   Note: If your sample is high in phenolics (see page 5) and you are using the **Phenolic Separation Solution**, reduce **Solution PD1 to 410 µl and add 40 µl of the Phenolic Separation Solution.**

   What's happening: Plant material is added to the Bead Tube to prepare it for a bead beating homogenization step. The PSS disassociates the phenolics from the nucleic acids so that they can be removed during the Inhibitor Removal Technology® (IRT) process.

2. Check **Solution PD2** for precipitates, if precipitated, warm at 37°C - 55°C until dissolved. Add 50 µl of **Solution PD2**.

   What's happening: Solution PD2 contains SDS. It will form a precipitate if it gets cold. Heating and dissolving the solids will restore it to full efficiency.

3. Add 3 µl of **RNase A Solution** to the PowerPlant® Bead Tube and vortex briefly to mix.

   What's happening: The RNase A will digest the unwanted RNA during the homogenization step.

4. Homogenize using one of the following methods:
   Note: See ***Heating of Samples Prior to Bead Beating*** in the **Hints and Troubleshooting Guide (page 13)**.

   A. Vortex:

   Secure PowerPlant® Bead Tubes horizontally using the MO BIO Vortex Adapter (MO BIO Catalog# 13000-V1-24) or on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
   Note: Most leaf tissues are soft and can be processed for DNA isolation by using a vortex adapter. However, plant tissues such as roots, wood, and plant seeds require pre-grinding with a mortar and pestle before placing on the vortex.

   B. PowerLyzer™ 24 Homogenizer:

   1. Properly identify each PowerPlant® Bead Tube on both the cap and on the side.

      Note: Due to the high energies of the PowerLyzer™ 24, the potential for marring of the cap tops 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 PowerPlant® 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 2 minutes.

Plant Tissue Type	Speed	No. of Cycles	Time/Cycle
Soft leaf tissues	2000 RPM	1	2 minutes
Fibrous leaf tissues	2200 RPM	1	2 minutes
Stems	2200 RPM	1	2 minutes

Plant Tissue Type	Speed	No. of Cycles	Time/Cycle
Roots	2500 RPM	1	2 minutes
Pine needles	2600 RPM	1	2 minutes
Seeds	2800 RPM	1	2 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.

What's happening: The bead beating step homogenizes plant material without the need for manual grinding. In some cases the plant material will not be completely disintegrated after the specified times of each method. However, there should be sufficient disruption for a good yield of DNA.

5. Centrifuge Bead Tubes at 13,000 x *g* for 2 minutes.

   What's happening: This step will pellet unwanted cell and tissue debris.

6. Transfer the supernatant to a clean **2 ml Collection Tube** (provided).
   Note: With 50 mg of plant tissue and depending upon plant type, expect 450 – 550 µl of supernatant, which may contain some particles.

   What's happening: The supernatant contains DNA and other cell components. Avoid transferring any solid plant tissue at this point.

7. Add 175 µl of **Solution PD3**. Vortex 5 seconds. Incubate at 4°C for 5 minutes.
   Note: For problematic samples you can add up to 250 µl of PD3 at this step. It is best to start at 175 µl with most sample types.

   What's happening: Solution PD3 is a novel formulation of Inhibitor Removal Technology® (IRT) and completes the process for removing PCR inhibitors in one step.

8. Centrifuge the Collection Tube for 2 minutes at 13,000 x *g.*

   What's happening: This step pellets the proteins and inhibitors.

9. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean **2 ml Collection Tube** (provided).

10. Add 600 µl of **Solution PD4** and 600 µl of **Solution PD6**. Vortex to mix for 5 seconds.

    What's happening: Solution PD4 is a binding salt. The concentration and amount of salt allows for optimal DNA binding to the silica spin filter membrane. Solution PD6 is an ethanol based buffer that allows for maximal nucleic acid binding to the column.

11. Load approximately 600 µl of lysate onto the **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds. Discard the flow through, place the Spin Filter back into the Collection Tube and add another 600 µl of lysate and centrifuge at 10,000 x *g* for 30 seconds. Discard the flow-through and repeat a third time until all of the lysate has been passed through the Spin Filter. Discard the flow-through and place the Spin Filter back into the Collection Tube.

    What's happening: In the presence of Solution PD4 & Solution PD6, DNA will bind to the spin filter. Centrifugation of the combined lysate through the spin filter allows the DNA to bind the filter membrane while allowing unwanted salt and impurities to pass through the membrane.

12. Add 500 µl of **Solution PD5** to the Spin Filter column. Centrifuge for 30 seconds at 10,000 x *g*. Discard the flow through. Place the Spin Filter back into the same Collection Tube.

    What's happening: Solution PD5 is an ethanol containing wash buffer that removes residual salt and other impurities from the spin filter membrane.

13. Add 500 µl of **Solution PD6** to the Spin Filter column. Centrifuge for 30 seconds at 10,000 x *g*. Discard the flow through. Place the Spin Filter back into the same Collection Tube.

    What's happening: Solution PD6 is an ethanol based buffer to completely remove all metabolites and salt from the spin filter membrane.

14. Centrifuge again for 2 minutes at up to 16,000 x *g* to remove residual **Solution PD6**.

    What's happening: This is a critical step. It is very important to remove all traces of the previous wash solutions before continuing.

15. Carefully place the **Spin Filter** into a new clean **2 ml Collection Tube** (provided). Avoid splashing any **Solution PD6** onto the **Spin Filter**.

16. Add 50 - 100 µl of **Solution PD7** (10 mM Tris, pH 8.0) to the center of the white filter membrane and incubate for 2 minutes at room temperature.

17. Centrifuge 30 seconds at 10,000 *x g*.
    Note: For maximum elution efficiency re-load the flow through once again to the center of the white filter membrane. Centrifuge 30 seconds at 10,000 *x g*.

    What's happening: Solution PD7 is 10 mM Tris, pH 8.0. The bound DNA is re-solubilized from the membrane into the low salt buffer that is neutral pH which protects DNA during storage.

18. Discard the **Spin Filter**. DNA in the tube is now ready to use. No further steps are required.

    We recommend storing DNA frozen (-20°C). **Solution PD7** contains no EDTA.

Thank you for choosing the PowerPlant® Pro DNA Isolation Kit.

Hints and Troubleshooting Guide

Heating of Samples Prior to Bead Beating
Some plant tissues may require heating of the sample in the presence of Solution PD1/Solution PD2 and the optional Phenolic Separation Solution at 65°C for 10 minutes just prior to the bead beating step. This varies dramatically between species and portion of the plant. We have found that most leaf, grass and pine needle samples do not need the heating step. Your specific sample will need to be assessed for optimization.

Amount of Bead Beating
Some plant tissues may require more or less bead beating from the recommended starting points stated on pages 8 & 10. This also varies dramatically between species and portion of the plant. Your specific sample will need to be assessed for optimization.

Concentrating the DNA
The final volume will be 50 -100 µl. If this is too dilute for your purposes, add 5 M NaCl to a final concentration of 0.2 M, or 3 M Sodium Acetate (pH: 5.2) to a final concentration of 0.3 M and mix. Then add two volumes of 100% cold ethanol. Mix by inversion and incubate at -20°C for 10 minutes to overnight. Centrifuge at 13,000 x *g* for 20 minutes to pellet the DNA. Decant the ethanol. Wash the DNA pellet with 70% ethanol (0.5 ml) by inversion. Centrifuge 10 minutes at 13,000 *x g*. Decant the ethanol and invert the tube to completely drain. Dry the residual ethanol in a speed vac, dessicator or ambient air. Resuspend the precipitated DNA in the desired volume.

Amount of Plant Tissue to Process
For fresh plant tissue, we recommend starting with 50 mg of tissue for most plant types. See Average DNA Yields (page 5) for guidelines on the average DNA yields for a variety of plant samples when starting with 50 mg of sample. For lyophilized or dried plant tissue we recommend using between 5-10 mg for most plant types.

DNA Floats Out of Well When Loaded on a Gel
You may have inadvertently transferred some residual Solution PD6 into the final sample. Prevent this by being careful in step 15 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation is the best way to remove residues of Solution PD6. (See Concentrating the DNA above.)

***Storing* DNA**
DNA eluted in Solution PD7 (10 mM Tris, pH 8.0) must be stored at -20°C. DNA can be eluted in TE (cat# 17325-1000) or TE-4 (10 mM Tris, 0.1 mM EDTA, cat# 17320-1000).

Contact Information

Technical Support:
Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: technical@mobio.com
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For the distributor nearest you, visit our web site at www.mobio.com/distributors

Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com

Description	Catalog No.	Quantity
PowerPlant® RNA Isolation Kit	13500-50	50 preps
PowerPlant® RNA Isolation Kit with DNase	13550-50	50 preps
PowerPlant® Pro-htp 96 Well DNA Isolation Kit	13496-4	4 x 96 preps
RTS DNase™ Kit	15200-50	50 preps
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
PowerLyzer™ 24 Bench Top Bead-Based Homogenizer	13155	1 unit