

# UltraClean<sup>®</sup> Microbial DNA Isolation Kit

| Catalog No. | Quantity  |
|-------------|-----------|
| 12224-50    | 50 Preps  |
| 12224-250   | 250 Preps |

# Instruction Manual

New protocol instruction: Shake Solution MD3 to mix before using to ensure consistent results.





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### Introduction

The UltraClean<sup>®</sup> Microbial DNA Isolation Kit is designed to isolate high-quality genomic DNA from microorganisms. A variety of microorganisms, including bacterial and fungal spores, have been tested successfully with this kit.

### **Protocol Overview**

Microbial cells, resuspended in bead solution are added to a bead beating tube containing beads, followed by lysis solution. The principal is to lyse the microorganisms by a combination of heat, detergent, and mechanical force against specialized beads. The cellular components are lysed by mechanical action using a specially designed MO BIO Vortex Adapter on a standard vortex. From the lysed cells, the released DNA is bound to a silica Spin Filter. The filter is washed, and the DNA is recovered in certified DNA-free Tris buffer.

## **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<sup>™</sup> Manifold allows for processing of up to 20 spin filter preps at a time using the PowerVac<sup>™</sup> Mini Spin Filter Adapters. The UltraClean<sup>®</sup>-htp 96 Well Microbial DNA Isolation Kit is available 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 bacteria, 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 <sup>®</sup> Microbial RNA Isolation Kit | 15800-50    | 50 preps             |
|   | 15800-250   | 250 preps            |
| UltraClean <sup>®</sup> PCR Clean-Up Kit            | 12500-50    | 50 preps             |
|   | 12500-100   | 100 preps            |
|   | 12500-250   | 250 preps            |
| UltraClean <sup>®</sup> -htp 96 Well Microbial DNA  | 10196-4     | 4 x 96 preps         |
| Isolation Kit                                       | 10196-12    | 12 x 96 preps        |
| PowerVac™ Manifold                                  | 11991       | 1 manifold           |
| PowerVac™ Mini System                               | 11992       | 1 unit + 20 adapters |
| PowerVac™ Mini Spin Filter Adapters                 | 11992-10    | 10 adapters          |
|   | 11992-20    | 20 adapters          |



# UltraClean<sup>®</sup> Microbial DNA Isolation Kit





## **Equipment Required**

Microcentrifuge (10,000 x g) Pipettor (50  $\mu$ l – 200  $\mu$ l, 100  $\mu$ l – 1000  $\mu$ l) Vortex-Genie<sup>®</sup> 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220) Vortex Adapter (MO BIO Catalog# 13000-V1)

## **Reagents Required but not Included**

100% ethanol (for the PowerVac<sup>™</sup> Manifold protocol only)

## Kit Contents

|                                  | Kit Catalog # 12224-50 |         | Kit Catalog # 12224-2 |           |
|----------------------------------|------------------------|---------|-----------------------|-----------|
| Component                        | Catalog #              | Amount  | Catalog #             | Amount    |
| MicroBead Tubes                  | 12224-50-BT            | 50      | 12224-250-BT          | 250       |
| (contain 250 mg MicroBeads)      |                        |         |                       |           |
| MicroBead Solution               | 12224-50-BS            | 16.5 ml | 12224-250-BS          | 80 ml     |
| Solution MD1                     | 12224-50-1             | 2.75 ml | 12224-250-1           | 15 ml     |
| Solution MD2                     | 12224-50-2             | 5.5 ml  | 12224-250-2           | 30 ml     |
| Solution MD3                     | 12224-50-3             | 50 ml   | 12224-250-3           | 250 ml    |
| Solution MD4                     | 12224-50-4             | 16.5 ml | 12224-250-4           | 3 x 32 ml |
| Solution MD5                     | 12224-50-5             | 3 ml    | 12224-250-5           | 15 ml     |
| Spin Filters Units in 2 ml Tubes | 12224-50-SF            | 50      | 12224-250-SF          | 250       |
| 2 ml Collection Tubes            | 12224-50-T             | 200     | 12224-250-T           | 1000      |

## **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 <u>www.mobio.com</u>. Reagents labeled flammable should be kept away from open flames and sparks.

**WARNING:** Solution MD4 contains ethanol, it is flammable. Do not use bleach to clean the inside of the PowerVac<sup>™</sup> Manifold or to rinse the PowerVac<sup>™</sup> Mini Spin Filter Adapters when attached to the manifold.

**IMPORTANT NOTE FOR USE:** Make sure the 2 ml MicroBead Tubes rotate freely in the centrifuge without rubbing. Do not spin the MicroBead Tubes in excess of 10,000 x g. Shake to mix Solution MD3 before use.



# **Experienced User Protocol**

## (If this is your first time using this kit please read the Detailed Protocol on the following page) Please wear gloves at all times

- Add 1.8 ml of microbial (bacteria, yeast) culture to a 2 ml Collection Tube (provided) and centrifuge at 10,000 x g for 30 seconds at room temperature. Decant the supernatant and spin the tubes at 10,000 x g for 30 seconds at room temperature and completely remove the media supernatant with a pipette tip. NOTE: Based on the type of microbial culture, it may be necessary to centrifuge longer than 30 seconds.
- 2. Resuspend the cell pellet in 300  $\mu$ l of **MicroBead Solution** and gently vortex to mix. Transfer resuspended cells to **MicroBead Tube**.
- 3. Check **Solution MD1.** If **Solution MD1** is precipitated, heat the solution at 60°C until the precipitate has dissolved. Add 50 μl of **Solution MD1** to the **MicroBead Tube**.
- 4. **Optional:** To increase yields, to minimize DNA shearing, or for difficult cells, see Alternative lysis methods in the "Hints & Troubleshooting Guide" section before continuing.
- 5. Secure **MicroBead Tubes** horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See "Hints & Troubleshooting Guide" for less DNA shearing).
- 6. Make sure the 2 ml **MicroBead Tubes** rotate freely in the centrifuge without rubbing. Centrifuge the tubes at 10,000 x g for 30 seconds at room temperature. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.
- 7. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
- 8. **NOTE**: Expect 300 to 350 µl of supernatant.
- 9. Add 100  $\mu$ l of **Solution MD2**, to the supernatant. Vortex for 5 seconds. Then 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 ml Collection Tube** (provided). Expect approximately 450 μl in volume.
- 12. Shake to mix Solution MD3 before use. Add 900  $\mu$ l of **Solution MD3** to the supernatant and vortex for 5 seconds.
- 13. Load about 700  $\mu$ l into the **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard the flow through, add the remaining supernatant to the **Spin Filter**, and centrifuge at 10,000 x *g* for 30 seconds at room temperature. **NOTE**: A total of 2 to 3 loads for each sample processed are required. Discard all flow through liquid.
- 14. Add 300 μl of **Solution MD4** and centrifuge at room temperature for 30 seconds at 10,000 x g.
- 15. Discard the flow through.
- 16. Centrifuge at room temperature for 1 minute at 10,000 x g.
- 17. Being careful not to splash liquid on the spin filter basket, place **Spin Filter** in a new **2 ml Collection Tube** (provided).
- 18. Add 50  $\mu$ l of **Solution MD5** to the center of the white filter membrane.
- 19. Centrifuge at room temperature for 30 seconds at 10,000 x g.
- 20. Discard **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°C). **Solution MD5** contains no EDTA.

# Thank you for choosing the UltraClean<sup>®</sup> Microbial DNA Isolation Kit.



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

1. Add 1.8 ml of microbial (bacteria, yeast) culture to a **2 ml Collection Tube** (provided) and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Decant the supernatant and spin the tubes at 10,000 x *g* for 30 seconds at room temperature and completely remove the media supernatant with a pipette tip.

What's happening: This step concentrates and pellets the microbial cells. In some cases it may take longer to completely pellet the cells. It is important to pellet the cells completely and remove all the culture media in this step.

2. Resuspend the cell pellet in 300 μl of **MicroBead Solution** and gently vortex to mix. Transfer resuspended cells to **MicroBead Tube**.

What's happening: The MicroBead Solution contains salts and a buffer which stabilizes and homogeneously disperses the microbial cells prior to lysis.

3. Check **Solution MD1.** If **Solution MD1** is precipitated, heat the solution at 60°C until the precipitate has dissolved. Add 50 μl of **Solution MD1** to the **MicroBead Tube**.

What's happening: Solution MD1 contains SDS and other disruption agents required for cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will precipitate. Heating at 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. In addition, Solution MD1 can be used while it is still warm.

4. **Optional:** To increase yields, to minimize DNA shearing, or for difficult cells, see Alternative lysis methods in the "Hints & Troubleshooting Guide" section before continuing.

What's happening: This optional step can lead to better performance in some cases. We recommend using only one of these methods for any individual prep.

5. Secure **MicroBead Tubes** horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See "Hints & Troubleshooting Guide" for less DNA shearing).

What's happening: This step creates the combined chemical/ mechanical lysis conditions required to release desired nucleic acids from microbial cells. Many cell types will not lyse without this chemically enhanced bead beating process. The vortex action is typically all that is required, however, more robust bead beaters may also be used. In most cases the times may be shorter with other devices but you may run the risk of increased DNA shearing. This process is compatible with fast prep machines.

6. Make sure the 2 ml **MicroBead Tubes** rotate freely in the centrifuge without rubbing. Centrifuge the tubes at 10,000 x g for 30 seconds at room temperature. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.

What's happening: The cell debris is sent to the bottom of the tube while DNA is remains in the supernatant.

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



8. NOTE: Expect 300 to 350 µl of supernatant.

What's happening: The volume to expect will vary depending on the size of the original cell pellet from step 1.

- Add 100 μl of Solution MD2, to the supernatant. Vortex for 5 seconds. Then incubate at 4°C for 5 minutes.
- 10. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.

What's happening: Solution MD2 contains a reagent to precipitate non-DNA organic and inorganic material including cell debris and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

11. Avoiding the pellet, transfer the entire volume of supernatant to a clean **2 ml Collection Tube** (provided). Expect approximately 450 μl in volume.

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

12. Shake to mix Solution MD3 before use. Add 900  $\mu$ l of **Solution MD3** to the supernatant and vortex for 5 seconds.

What's happening: Solution MD3 is a highly concentrated salt solution. It sets up the high salt condition necessary to bind DNA to the Spin Filter membrane in the following step.

13. Load about 700 μl into the Spin Filter and centrifuge at 10,000 x g for 30 seconds at room temperature. Discard the flow through, add the remaining supernatant to the Spin Filter, and centrifuge at 10,000 x g for 30 seconds at room temperature. NOTE: A total of 2 to 3 loads for each sample processed are required. Discard all flow through liquid.

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

14. Add 300 μl of **Solution MD4** and centrifuge at room temperature for 30 seconds at 10,000 x g.

What's happening: Solution MD4 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 residues of salt, and other contaminants while allowing the DNA to stay bound to the silica membrane.

15. Discard the flow through.

What's happening: This flow through is waste containing ethanol wash solution and contaminants that did not bind to the silica Spin Filter membrane.

16. Centrifuge at room temperature for 1 minute at 10,000 x g.

What's happening: This step removes residual Solution MD4 (ethanol wash solution). It is critical to remove all traces of wash solution because it can interfere with down stream DNA applications.

17. Being careful not to splash liquid on the spin filter basket, place **Spin Filter** in a new **2 ml Collection Tube** (provided).



What's happening: It is important to avoid any traces of the ethanol based wash solution.

18. Add 50  $\mu$ l of **Solution MD5** to the center of the white filter membrane.

What's happening: Placing the Solution MD5 (elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in more efficient release of bound DNA

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

What's happening: As the Solution MD5 (elution buffer) passes through the silica membrane, DNA is released, and it flows through the membrane, and into the Collection Tube. The DNA is released because it can only bind to the silica Spin Filter membrane in the presence of salt. Solution MD5 is 10mM Tris pH 8 and does not contain salt.

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

We recommend storing DNA frozen (-20°C). Solution MD5 contains no EDTA.

Thank you for choosing the UltraClean<sup>®</sup> Microbial DNA Isolation Kit.



# Vacuum Protocol using the PowerVac<sup>™</sup> Manifold Please wear gloves at all times

For each sample lysate, use one Spin Filter column. Keep the Spin Filter in the attached 2 ml Collection Tube and continue using the Collection Tube as a Spin Filter holder until needed for the Vacuum Manifold Protocol. Label each Collection Tube top and Spin Filter column to maintain sample identity. If the Spin Filter becomes clogged during the vacuum procedure, you can switch to the procedure for purification of the DNA by centrifugation.

You will need to provide 100% ethanol for step 4 of this protocol

- For each prep, attach one aluminum PowerVac<sup>™</sup> Mini Spin Filter Adapter (MO BIO Catalog# 11992-10 or 11992-20) into the Luer-Lok® fitting of one port in the manifold. Gently press a Spin Filter column into the PowerVac<sup>™</sup> Mini Spin Filter Adapter until snugly in place. Ensure that all unused ports of the vacuum manifold are closed. Note: Aluminum PowerVac<sup>™</sup> Mini Spin Filter Adapters are reusable.
- 2. Transfer 650 µl of prepared sample lysate (from step 12) to the Spin Filter column.
- 3. Turn on the vacuum source and open the stopcock of the port. Hold the tube in place when opening the stopcock to keep the spin filter steady. Allow the lysate to pass through the Spin Filter column. After the lysate has passed through the column completely, load again with the next 650 μl of lysate. Continue until all of the lysate has been loaded onto the Spin Filter column. Close the one-way Luer-Lok® stopcock of that port.

**Note:** If Spin Filter Columns are filtering slowly, close the ports to samples that have completed filtering to increase the pressure to the other columns.

- Load 800 μl of 100% ethanol into the Spin Filter so that it completely fills the column. Open the stopcock while holding the column steady. Allow the ethanol to pass through the column completely. Close the stopcock.
- 5. Add 300 μl of **Solution MD4** to each Spin Filter. Open the Luer-Lok® stopcock and apply a vacuum until **Solution MD4** has passed through the Spin Filter completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.
- 6. Turn off the vacuum source and open an unused port to vent the manifold. If all 20 ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.
- 7. Remove the **Spin Filter column** and place in the original labeled **2 ml Collection Tube**. Place into the centrifuge and spin at  $13,000 \times g$  for 1 minute to completely dry the membrane.
- Transfer the Spin Filter column to a new 2 ml Collection Tube and add 100 μl of Solution MD5 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).
- 9. Centrifuge at room temperature for 30 seconds at 10,000 x g.



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

We recommend storing DNA frozen (- $20^{\circ}$  to - $80^{\circ}$ C). **Solution MD5** contains no EDTA. To concentrate the DNA see the Hints & Troubleshooting Guide.

Thank you for choosing the UltraClean<sup>®</sup> Microbial DNA Isolation Kit.



# Hints and Troubleshooting Guide

Alternative Lysis Methods (We recommend using only one of these methods for any individual prep.)

- **To increase yields**: Heating can aid in lysis for some organisms and it can lead to increased yields. Heat preps at 65°C for 10 minutes and continue with step 5.
- For less DNA shearing: We recommend heating the preps at 65°C for 10 minutes with occasional bump vortexing for a few seconds every 2-3 minutes. Skip step 5 and go to step 6. This helps prevent unwanted damage to large DNA. This procedure will reduce DNA shearing and at the same time can increase the yield of total DNA for some organisms.
- If cells are difficult to lyse: Heat the preps at 70°C for 10 minutes. Follow by continuing with the protocol at step 5.

## Concentrating the DNA

The final volume of eluted DNA will be 50  $\mu$ l. The DNA may be concentrated by adding 5  $\mu$ l of 5M NaCl and inverting 3-5 times to mix. Next, add 100  $\mu$ l of 100% cold ethanol and invert 3-5 times to mix. Incubate at -20°C for 30 minutes and centrifuge at 10,000 x *g* for 15 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac or dessicator or air dry. Resuspend precipitated DNA in sterile water or Solution MD5 (10 mM Tris).

## DNA Floats Out of Well When Loaded on a Gel

This usually occurs because residual Solution MD4 remains in the final sample. Prevent this by being careful in step 17 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 MD4.

## Storing DNA

DNA is eluted in Solution MD5 (10 mM Tris) and must be stored at -20°C to -80°C to prevent degradation. For long term storage, we recommend aliquoting DNA into appropriate volumes and store at -80°C. DNA can be eluted in TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-Free PCR Grade Water (MO BIO Catalog# 17000-10).

## Cleaning of the PowerVac™ Mini Spin Filter Adapters

It is recommended to rinse the PowerVac<sup>™</sup> Mini Spin Filter Adapters promptly after use to avoid salt build up. To clean the PowerVac<sup>™</sup> Mini Spin Filter Adapters, rinse each adapter with DI water followed by 70% ethanol and flush into the manifold base. Alternatively, remove the adapters and wash in laboratory detergent and DI water. PowerVac<sup>™</sup> Mini Spin Filter Adapters may be autoclaved.

Do not use bleach to clean the PowerVac<sup>™</sup> Mini Spin Filter Adapters while attached to the PowerVac<sup>™</sup> Manifold. Bleach should never be mixed with solutions containing guanidine and should not be used to clean the PowerVac<sup>™</sup> Manifold. For more information on cleaning the PowerVac<sup>™</sup> Manifold, please refer to the PowerVac<sup>™</sup> Manifold manual.



# **Contact Information**

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

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

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



| DNA Purification and Gel Extraction                 | Catalog No. | Quantity                      | RNA Is         |
|---|-------------|-------------------------------|----------------|
| PowerClean® DNA Clean-Up Kit                        | 12877-50    | 50 preps                      | UltraC         |
| UltraClean® 15 DNA Purification Kit                 | 12100-300   | 300 preps                     | Genor          |
| UltraClean® PCR Clean-Up Kit                        | 12500-50    | 50 preps                      | Power          |
|   | 12500-100   | 100 preps                     | Kit            |
| Liltra Claar® http://wall.BCB.Claar                 | 12500-250   | 250 preps                     | <b>Di</b> Ooti |
| Un Kit  | 12596-4     | 4 x 96 preps<br>12 x 96 preps | Kit            |
| UltraClean® GelSpin® DNA                            | 12400-50    | 50 preps                      | Bi Ostic       |
| Extraction Kit                                      | 12400-100   | 100 preps                     | Kit            |
|   | 12400-250   | 250 preps                     |                |
| Plasmid DNA Isolation                               | Catalog No. | Quantity                      | Bi Ostio       |
| UltraClean® 6 Minute Mini Plasmid                   | 12300-50    | 50 preps                      | Power          |
| Prep Kit  | 12300-100   | 100 preps                     |                |
| Liltra Cloan® Standard Mini Blasmid                 | 12300-250   | 250 preps                     | Power          |
| Pren Kit  | 12301-50    | 100 preps                     | FOwer          |
|   | 12301-250   | 250 preps                     |                |
| UltraClean®-htp 96 Well Plasmid Prep                | 12396-4     | 4 x 96 preps                  | Power          |
| Kit   | 12396-12    | 12 x 96 preps                 | Isolatio       |
| UltraClean® Midi Plasmid Prep Kit                   | 12700-20    | 20 preps                      | UltraC         |
|   | 12/00-50    | 50 preps                      | 1.111 0        |
| UltraClean® Maxi Plasmid Prep Kit                   | 12600-10    | 20 preps                      | UltraC         |
| UltraClean® Endotoxin-Free Mini                     | 12311-100   | 100 preps                     | IltraC         |
| Plasmid Prep Kit                                    | 12311-250   | 250 preps                     | Kit            |
| UltraClean® Endotoxin-Free Midi<br>Plasmid Prep Kit | 12711-10    | 10 preps                      | Power          |
| UltraClean® Endotoxin-Free Maxi<br>Plasmid Prep Kit | 12611-10    | 10 preps                      | UltraC         |
| UltraClean® Endotoxin Removal Kit                   | 12615       | 1 kit                         | Power          |
| UltraClean® Endotoxin-Free Ethanol                  | 12616       | 1 kit                         | Power          |
| Precipitation Kit                                   |             |                               | Kit            |
| UltraClean® Endotoxin Removal                       | 12625-25    | 25 ml                         | UltraC         |
| Reagent   | 40000.45    | 45 mal                        | Kit            |
| Endotoxin-Free Sodium Chionde                       | 12020-15    | 10 mi                         |                |
| Endotoxin-Free Centrifuge Tubes                     | 12617-100   | 100 each/2 ml                 | Power          |
|   | 12618-50    | 50 each/15 ml                 |                |
|   | 12010-30    | tubes                         |                |
|   | 12619-25    | 25 each/50 ml                 |                |
|   |             | tubes                         |                |
| RNA Isolation                                       | Catalog No. | Quantity                      | UltraC         |
| LifeGuard <sup>™</sup> Soil Stabilization Solution  | 12868-10    | 10 ml                         | UltraC         |
|   | 12868-100   | 100 ml                        | Isolatio       |
|   | 12868-1000  | 1 L                           |                |
| On Spin Column DNaco L Kit (DNaco                   | 12868-7500  | 7.5 L                         | L litro Cl     |
| Free)   | 10100-00    | ou preps                      | Isolatio       |
| BiOstic® Stabilized Blood RNA                       | 12231-20    | 20 preps                      | UltraC         |
| Isolation Kit                                       | 12231-50    | 50 preps                      | Isolatio       |
| PiOtic® Placed Total PNA location                   | 12231-100   | 20 props                      | Lilltra Cl     |
| Riter Blood Total KINA Isolation                    | 12230-20    | 20 preps                      | (Non-S         |
| RNA PowerSoil® DNA Flution                          | 12867-25    | 25 preps                      | UltraC         |
| Accessory Kit                                       |             | -0 p. 0p0                     | (Proce         |
| RNA PowerSoil® Total RNA Isolation                  | 12866-25    | 25 preps                      | UltraC         |
| Kit   |             |                               | Plus R         |
|   | 45000.50    |                               | (Proce         |
| UltraClean® Microbial RNA Isolation                 | 15800-50    | 50 preps                      | UltraC         |
|   | 15800-250   | ∠ou preps                     | ISOlatio       |
| Unraclearter Issue & Cells RNA<br>Isolation Kit     | 15000-250   | 250 preps                     |                |
| ISUICIUUI INI                                       | 10000 200   | 200 01000                     | DINA IS        |

| RNA Isolation Continued                        | Catalog No. | Quantity      |
|--|-------------|---------------|
| UltraClean® Plant RNA Isolation Kit            | 13300-20    | 20 preps      |
|  | 13300-50    | 50 preps      |
| Genomic DNA Isolation                          | Catalog No. | Quantity      |
| PowerFood <sup>™</sup> Microbial DNA Isolation | 21000-50    | 50 preps      |
| Kit  | 21000-100   | 100 preps     |
|  |             |               |
| BiOstic® Bacteremia DNA Isolation              | 12240-50    | 50 preps      |
| Kit  |             |               |
| BiOstic® FFPE Tissue DNA Isolation             | 12250-50    | 50 preps      |
| Kit  |             |               |
|  |             |               |
| BiOstice Paraffin Removal Reagent              | 12251-50    | 2 x 25 ml     |
|  | 12000 10    | 10 propo      |
| PowerMax® Soil DNA Isolation Kit               | 12900-10    | to preps      |
|  |             |               |
| PowerSoil® DNA Isolation Kit                   | 12888-50    | 50 preps      |
|  | 12888-100   | 100 preps     |
|  | 12000 100   | 100 0.000     |
| PowerSoil®-htp 96 Well Soil DNA                | 12955-4     | 4 x 96 preps  |
| Isolation Kit                                  | 12955-12    | 12 x 96 preps |
| UltraClean® Soil DNA Isolation Kit             | 12800-50    | 50 preps      |
|  | 12800-100   | 100 preps     |
| UltraClean®-htp 96 Well Soil DNA               | 12896-4     | 4 x 96 preps  |
| Isolation Kit                                  | 12896-12    | 12 x 96 preps |
| UltraClean® Mega Soil DNA Isolation            | 12900-10    | 10 preps      |
| Kit  |             | - 1 - 1 -     |
| PowerClean® DNA Clean-Up Kit                   | 12877-50    | 50 preps      |
|  |             |               |
| UltraClean® Fecal DNA Isolation Kit            | 12811-50    | 50 preps      |
|  | 12811-100   | 100 preps     |
| PowerMicrobial® Midi DNA Isolation             | 12225-25    | 25 preps      |
| Kit  |             |               |
| PowerMicrobial® Maxi DNA Isolation             | 12226-25    | 25 preps      |
| Kit  |             |               |
| UltraClean® Microbial DNA Isolation            | 12224-50    | 50 preps      |
| Kit  | 12224-250   | 250 preps     |
| UltraClean®-htp 96 Well Microbial              | 10196-4     | 4 x 96 preps  |
| DNA Isolation Kit                              | 10196-12    | 12 x 96 preps |
| PowerPlant® DNA Isolation Kit                  | 13200-50    | 50 preps      |
|  | 13200-100   | 100 preps     |
|  |             |               |
|  |             |               |
|  |             |               |
| IlltraClean® Plant DNA Isolation Kit           | 13000-50    | 50 preps      |
| Characteanter name DIVA Isolation All          | 13000-250   | 250 preps     |
| UltraClean®-htp 96 Well Plant DNA              | 13096-4     | 4 x 96 preps  |
| Isolation Kit                                  | 13096-12    | 12 x 96 preps |
|  |             |               |
|  |             |               |
| UltraClean® Tissue & Cells DNA                 | 12334-50    | 50 preps      |
| Isolation Kit                                  | 12334-250   | 250 preps     |
| UltraClean®-htp 96 Well Tissue DNA             | 12996-4     | 4 x 96 preps  |
| Isolation Kit                                  | 12996-12    | 12 x 96 preps |
|  |             |               |
| UltraClean® Blood DNA Isolation Kit            | 12000-100   | 100 preps     |
| (Non-Spin)                                     |             |               |
| UltraClean® Blood DNA Isolation Kit            | 12000-1000  | 1 kit         |
| (Processes 1,000 ml of Blood)                  | 10000 (000  | 4.1.11        |
| UltraClean® Blood DNA Isolation Kit            | 12002-1000  | 1 kit         |
| Plus RNase                                     |             |               |
| (Processes 1,000 ml of Blood)                  | 10000 50    | 50 preps      |
| UltraClean® BloodSpin® DNA                     | 12200-50    | ou preps      |
|  | 12200-250   | 250 preps     |
| UltraClean®-htp 96 Well BloodSpin®             | 12290-4     | 4 X 90 preps  |
| DINA ISOIATION KIT                             | 12230-12    | 1∠ x 90 preps |



| Genomic DNA Isolation                     |              | Overstitu              |
|---|--------------|------------------------|
| Continued                                 | 14000-10     | 10 isolations          |
| Kit                                       | 14000-20     | 20 isolations          |
| PowerWater® DNA Isolation Kit             |              | 50 preps               |
|   | 14900-50-NF  | (No filters)           |
|   | 14900-50-22  | (0.22 µm)              |
|   | 14900-50-45  | (0.45 µm)              |
|   | 14900-100-NF | (No filters)           |
|   | 14900-100-22 | (0.22  µm)             |
|   | 14900-100-45 | (0.45 µm)              |
| RapidWater <sup>™</sup> DNA Isolation Kit |              | 50 preps               |
|   | 14810-50-NF  | (No filters)           |
|   | 14810-50-22  | (0.22 µm)              |
|   | 14810-50-45  | (0.45 µm)<br>100 props |
|   | 14810-100-NF | (No filters)           |
|   | 14810-100-22 | (0.22 µm)              |
|   | 14810-100-45 | (0.45 µm)              |
| UltraClean® Water DNA Isolation Kit       | 14800-10     | 10 preps               |
| (0.45µm filters)                          | 14800-25     | 25 preps               |
|   | 4 4000 40    | 10                     |
| UltraClean® Water DNA Isolation Kit       | 14880-10     | 10 preps               |
| (∪.∠∠ µm miters)                          | 14000-20     | zo preps               |
| UltraClean® Water DNA Isolation Kit       | 14800-10-NF  | 10 preps               |
| (No filters)                              | 14800-25-NF  | 25 preps               |
|   |              | - 1 - 1 -              |
|   |              |                        |
|   |              | -                      |
| Microbiological Culture Media             | Catalog No.  | Quantity               |
| IB DRY®Powder Growth Media                | 12105-05     | 500 g                  |
|   | 12105-5      | 5 kg                   |
| LB Broth Powder Growth Media. pH          | 12106-05     | 500 g                  |
| 7   | 12106-1      | 1 kg                   |
|   | 12106-5      | 5 kg                   |
| LB Agar Powder Growth Media, pH 7         | 12107-05     | 500 g                  |
|   | 12107-1      | 1 kg                   |
| I B Broth (Lannay) Douvdar Crowth         | 12107-5      | 5 Kg                   |
| Media pH 7                                | 12108-05     | 500 g<br>1 ka          |
|   | 12108-5      | 5 kg                   |
| LB Agar (Lennox) Powder Growth            | 12109-05     | 500 g                  |
| Media, pH 7                               | 12109-1      | 1 kg                   |
|   | 12109-5      | 5 kg                   |
| Soybean-Casein Digest Medium              | 12114-05     | 500 g                  |
| (138), USP                                | 12114-1      | 1 Kg                   |
| Sovhaan-Casein Digest Agar                | 12114-0      | 5 Kg                   |
| Medium (TSA) LISP                         | 12115-05     | 500 g<br>1 ka          |
|   | 12115-5      | 5 kg                   |
|   |              | Ŭ.                     |
| Yeast Extract                             | 12110-05     | 500 g                  |
|   | 12110-1      | 1 kg                   |
|   | 12110-0      | эку                    |
|   |              |                        |
| Tryptone                                  | 12111-05     | 500 g                  |
|   | 12111-1      | 1 kg                   |
|   | 12111-5      | 5 kg                   |
| Agar, Bacteriological Grade               | 12112-05     | 500 g                  |
|   | 12112-1      | 1 kg                   |
| Other Reagents and Lab                    | 12112-0      | 5 Kg                   |
| Accessories                               | Catalog No.  | Quantity               |
| 20 bp DNA Ladder                          | 17020-40     | 40 µg                  |
|   | -            |                        |
|   |              |                        |

| Other Reagents and Lab                  |             |                |
|---|-------------|----------------|
| AccessoriesContinued                    | Catalog No. | Quantity       |
| 100 bp DNA Ladder                       | 17100-40    | 40 µg          |
|   | 47000 400   | 400            |
| 1 kb DNA Ladder                         | 17200-100   | 100 µg         |
|   |             |                |
|   |             |                |
|   |             |                |
|   |             |                |
|   |             |                |
|   | 45000 50    | 50             |
| UltraClean® Agarose, Molecular          | 15003-50    | 50 g           |
| Biology Grade                           | 15003-500   | 500 g          |
|   | 15003-1000  | 1 kg           |
|   |             |                |
|   |             |                |
|   |             |                |
|   | 15515-50    | 50 g           |
| Ultracilearity INIS-8 Agarose           | 15515-100   | 100 g          |
|   | 15515-500   | 500 g          |
| UltraClean® Forensic Agarose            | 15505-50    | 50 g           |
| , i i i i i i i i i i i i i i i i i i i | 15505-100   | 100 g          |
| -                                       | 15505-500   | 500 g          |
| UltraClean® Low Melt Agarose            | 15005-50    | 50 g           |
|   | 15005-100   | 100 g          |
| Litra Clean® Low Malt Sieve Agarosa     | 15005-500   | 500 g          |
| OllaClear® Low Men Sieve Agaiose        | 15004-100   | 100 g          |
|   | 15004-500   | 500 g          |
| Ethidium Bromide Solution               | 15006-1     | 1 ml           |
|   | 15006-10    | 10 ml          |
| Ethidium Drawida Dastaining Tas         | 45007.05    | 05 h a m       |
| Ethidium Bromide Destaining Tea         | 15007-25    | 25 bags        |
| Days                                    |             |                |
| Bromophenol Blue Gel Loading            | 15008-1     | 1 ml           |
| Buffer                                  | 15008-5     | 5 x 1 ml       |
|   |             |                |
| Bromophenol Blue/Xylene Cyanol          | 15009-1     | 1 ml           |
| Gei Loading Buffer                      | 15009-5     | 5 X 1 MI       |
| TAE Buffer 50X (Tris-acetate-EDTA)      | 15001-100   | 100 ml         |
|   | 15001-500   | 500 ml         |
|   | 15001-1000  | 1 liter        |
| TBE Buffer, 10X (Tris-borate-EDTA)      | 15002-100   | 100 ml         |
|   | 15002-500   | 500 ml         |
| DNIago Free Claves                      | 15002-1000  | 1 liter        |
| RINASE-FILE GIUVES                      | 1555-5      | bag of 100     |
|   | 1555-M      | bag of 100     |
|   | 1555-L      | bag of 100     |
| UltraClean® Lab Cleaner                 | 12095-250   | 250 ml         |
|   | 40007 707   | squeeze bottle |
|   | 12095-500   | 500 ml spray   |
|   | 12005-1000  | 1 liter bottle |
| OmniTag™ DNA Polvmerase                 | 1224-250    | 250 reactions  |
| Enzyme                                  |             | (10 U/µl)      |
|   |             |                |
| OmniTaq™ DNA Polymerase 2x              | 1226-250    | 250 reactions  |
| Master Mix                              |             | (5 x 1.25      |
| Omni KlonTogIM DNA Dolymoroco           | 1225-250    | 250 reactions  |
|   | 1220-200    | (25 U/ul)      |
| Omni KlenTag™ DNA Polvmerase 2x         | 1227-250    | 250 reactions  |
| Master Mix                              |             | (5 x 1.25      |
|   |             | ml/tube)       |



| Other Reagents and Lab          |             |                  | Instrumentation and                   |                          |                 |
|---------------------------------|-------------|------------------|---------------------------------------|--------------------------|-----------------|
| AccessoriesContinued            | Catalog No. | Quantity         | Accessories Continued                 | Catalog No.              | Quantity        |
| DNase (RNase-Free)              | 15600-5     | 5 mg             | Glass 50 ml Bead Tubes, 0.1 mm        | 13145-10                 | 10 tubes        |
|                                 | 15601-100   | 2500 units       |                                       | 13145-50                 | 50 tubes        |
|                                 |             |                  |                                       | 13145-100                | 500 tubes       |
| Proteinase K                    | 1223-100    | 100 mg           | Glass 15 ml Bead Tubes, 1.0 mm        | 13136-50                 | 50 tubes        |
|                                 | 1222-2      | 2 ml (20         |                                       | 1010000                  | 0010000         |
|                                 |             | mg/mÌ)           |                                       |                          |                 |
| Ribonuclease A (25 mg/ml)       | 1202-1      | 1 ml             | Ceramic 15 ml Bead Tubes, 1.4 mm      | 13137-50                 | 50 tubes        |
| DOD Weter                       | 1202-5      | 5 ml             | Oceanic 50 ml Decid Tables 4.4 mm     | 4044740                  | 40 to be a -    |
| PCR water                       | 17000-1     | 1 mi<br>5 x 1 ml | Ceramic 50 mi Bead Tubes, 1.4 mm      | 13147-10                 | 10 tubes        |
|                                 | 17000-10    | 10 x 1 ml        |                                       | 13147-50                 | 50 lubes        |
|                                 | 17000-11    | 10 ml bottle     |                                       |                          |                 |
| Molecular Biology Grade Water   | 17012-200   | 200 ml           | Metal 50 ml Bead Tubes, 2.38 mm       | 13149-10                 | 10 tubes        |
|                                 | 17012-5200  | 5 x 200 ml       |                                       | 13149-50                 | 50 tubes        |
| DEPC Treated Water              | 17011-200   | 200 ml           | PowerMix 15 ml Bead Tubes             | 13138-50                 | 50 tubes        |
|                                 | 17011-5200  | 5 X 200 mi       |                                       |                          |                 |
|                                 |             |                  |                                       |                          |                 |
| Endotoxin-Free Water            | 17013-10    | 10 ml            | PowerMix 50 ml Bead Tubes             | 13148-10                 | 10 tubes        |
|                                 | 17013-50    | 50 ml            |                                       | 13148-50                 | 50 tubes        |
|                                 | 17013-100   | 100 ml           |                                       |                          |                 |
|                                 | 17013-500   | 500 mi           | 2 ml Collection Tubes                 | 1200-100-T               | 100 tubes       |
|                                 |             |                  |                                       | 1200-100-1<br>1200-150-T | 150 tubes       |
| Instrumentation and Accessories | Catalog No. | Quantity         |                                       | 1200-250-T               | 250 tubes       |
| BagMixer® 400 VW                | 23112       | 1 unit           | 2 ml Screw Cap Tubes                  | 12800-200-E              | 200 tubes &     |
|                                 |             |                  |                                       |                          | caps            |
| BagFilter® 400 P                | 23113-500   | Box of 500       | 15 ml Collection Tubes                | 12700-T                  | 25 tubes        |
| Des Des 200 400                 | 00444.500   | Day of 500       | 50 ml Oantrifung Talans               | 40000 T                  | 05 tub se       |
| BagPage® 400                    | 23114-500   | Box of 500       | 50 mi Centrifuge Tubes                | 12600-T                  | 25 tubes        |
| Precellys®24 Homogenizer 120V   | 13112       | 1 unit           | Spin Filters (in 1.9 ml tubes)        | 1200-50-SE               | 50 filters      |
| Trecenys@24 Homogenizer, 1200   |             |                  |                                       | 1200-100-SF              | 100 filters     |
|                                 |             |                  |                                       | 1200-250-SF              | 250 filters     |
| Ceramic Bead Tubes, 1.4 mm      | 13113-50    | 50 bead tubes    | Endotoxin-Free Centrifuge Tubes       | 12617-100                | 100 each/2 ml   |
|                                 |             |                  |                                       |                          | tubes           |
|                                 |             |                  |                                       | 12618-50                 | 50 each/15 ml   |
|                                 |             |                  |                                       | 12619-25                 | 25 each/50 ml   |
|                                 |             |                  |                                       | .2010 20                 | tubes           |
| Ceramic Bead Tubes, 2.8 mm      | 13114-50    | 50 bead tubes    | 15 ml Midi Spin Filters               | 12700-SF                 | 25 spin filters |
| Glass Bead Tubes, 0.5 mm        | 13116-50    | 50 bead tubes    | Vortex-Genie® 2 Vortex (120V)         | 13111-V                  | 1 unit          |
| Glass Bead Tubes, 0.1 mm        | 13118-50    | 50 bead tubes    | Vortex-Genie® 2 Vortex (220V)         | 13111-V-220              | 1 unit          |
| Metal Bead Tubes. 2.38 mm       | 13117-50    | 50 bead tubes    | Vortex Adapter, holds 12 (1.5-2.0 ml) | 13000-V1                 | 1 unit          |
|                                 |             |                  | tubes                                 |                          |                 |
| 2.0 ml Tough Tubes with Cap     | 13119-500   | 500              | Vortex Adapter, holds 6 (5 ml) tubes  | 13000-V1-5               | 1 unit          |
|                                 | 13119-1000  | 1000             |                                       |                          |                 |
| Carbide Bead Tubes, 0.25 mm     | 13121-50    | 50 x 0.5 ml      | Vortex Adapter, holds 4 (15 ml) tubes | 13000-V1-15              | 1 unit          |
| Garnet Bead Tubes 0.15 mm       | 13122-50    | 50 x 0.5 ml      | Vortex Adapter, holds 2 (50 ml) tubes | 13000-\/1-50             | 1 unit          |
| Carrier Dead Tubes, 0.15 min    | 10122-00    | tubes            | Voltex Adapter, holds 2 (50 mi) tubes | 10000-01-00              | 1 Grint         |
| Garnet Bead Tubes, 0.70 mm      | 13123-50    | 50 x 2 ml        | Vortex Adapter, holds 24 (1.5-2.0 ml) | 13000-V1-24              | 1 unit          |
|                                 |             | tubes            | tubes                                 |                          |                 |
| Garnet + ¼ Ceramic 15 ml Bead   | 13134-50    | 50 tubes         | Power Supply w/Timer, (120V)          | 16023                    | 1 unit          |
| Carpet L 1/ Carpets 50 ml Bood  | 12144 10    | 10 tubes         | Bower Supply w/Timer (220)/)          | 16022 220                | 1 unit          |
| Tubes 0.70 mm `                 | 13144-10    | 50 tubes         | Power Supply w/ Timer, (220V)         | 10023-220                | i unit          |
|                                 | 13144-100   | 100 tubes        |                                       |                          |                 |
|                                 | 13144-500   | 500 tubes        |                                       |                          |                 |
| Glass 15 ml Bead Tubes, 0.1 mm  | 13135-50    | 50 tubes         | Polycarbonate Single-sided Comb       | 16005                    | 1 mm x 3 well   |
|                                 |             |                  |                                       | 16006                    | 1 mm x 8 well   |
|                                 |             |                  |                                       | 16007                    | 1 mm x 10 well  |
|                                 |             |                  |                                       | 16008                    | 1 mm x 12 well  |



| Instrumentation and                 |             |                |
|-------------------------------------|-------------|----------------|
| Accessories Continued               | Catalog No. | Quantity       |
| Polycarbonate Dual-sided Comb       | 16013       | 1 mm x 8       |
|                                     |             | well/16 well   |
|                                     | 16014       | 1 mm x 10      |
|                                     |             | well/14 well   |
|                                     | 16015       | 2 mm x 8       |
|                                     |             | well/16 well   |
|                                     | 16016       | 2 mm x 10      |
|                                     |             | well/14 well   |
| Teflon Single-sided Comb            | 16009       | 1 mm x 3 well  |
|                                     | 16010       | 1 mm x 8 well  |
|                                     | 16011       | 1 mm x 10 well |
|                                     | 16012       | 1 mm x 12 well |
| Teflon Dual-sided Comb              | 16017       | 1 mm x 8       |
|                                     |             | well/16 well   |
|                                     | 16018       | 1 mm x 10      |
|                                     |             | well/14 well   |
|                                     | 16019       | 2 mm x 8       |
|                                     | 40000       | well/16 well   |
|                                     | 16020       | 2 mm x 10      |
|                                     |             | well/14 well   |
| Mini Horizontal Gel System          | 16001       | 1 each         |
| Mini Horizontal Gel Caster, 3 place | 16003       | 1 each         |
| Mini Horizontal Gel Tray            | 16004       | 1 each         |
| 96 Well Plate Shaker (120V)         | 11996       | 1 unit         |

| Instrumentation and         |             |          |
|-----------------------------|-------------|----------|
| Accessories Continued       | Catalog No. | Quantity |
| 96 Well Plate Shaker (220V) | 11996-220   | 1 unit   |
| Plate Adapter Set           | 11999       | 1 set    |
| Tube Adapter Set            | 11995       | 1 set    |
| Vacuum Pump (120V)          | 11998       | 1 unit   |
| Vacuum Pump (220V)          | 11998-220   | 1 unit   |
| UltraVac™ Manifold          | 11997       | 1 unit   |
|                             |             |          |