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Preface

This preface contains:

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Safety

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—IMPORTANT, CAUTION, WARNING, DANGER—implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

⚠️ CAUTION – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠️ WARNING – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

⚠️ DANGER – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

⚠️ WARNING CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death. Contact your Environmental Health & Safety Department (EH&S) with any concerns or questions.
Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. See “About SDSs.”
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended on the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click Search.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

**Note:** For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

---

**Chemical Waste Hazard**

⚠️ **WARNING**  **CHEMICAL WASTE HAZARD.** Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death. If you have questions or concerns about a chemical waste hazard, contact your Environmental Health and Safety department.

---

**Chemical Waste Safety Guidelines**

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

**Biological Hazard Safety**

⚠️ **WARNING** BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* ([http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm)).
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; [http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)).
- Your company’s/institution’s Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at: [http://www.cdc.gov](http://www.cdc.gov)
How to Obtain More Information

Related Documentation
See the following related document for more information on the topics in this guide:

- PrepMan® Ultra Sample Preparation Quick Reference Card (PN 4367551)
- RapidFinder™ Online Help
- RapidFinder™ Quick Reference Card (PN 4366739)
- RapidFinder™ Installation User Bulletin (PN 4367395)

Send Us Your Comments
Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to http://www.appliedbiosystems.com, then click the link for Support. See “How to Obtain Support.”

How to Obtain Support
For the latest services and support information for all locations, go to http://www.appliedbiosystems.com, then click the link for Support.

At the Support page, you can:

- Obtain worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, SDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches
PrepMan Ultra Sample Preparation Reagent Protocol

This protocol covers:

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Preventing Contamination.............................................. 14
Prepare and Extract Samples for Bacterial and Fungal Testing ... 16
Prepare and Extract Food Samples for Pathogen Testing .......... 21
Prepare and Extract Food Samples for GMO Testing ............. 25
Appendix A - Filtration Methods ..................................... 28
Appendix B - Troubleshooting ......................................... 32
About the PrepMan Ultra Sample Preparation Reagent

Purpose of PrepMan Ultra Sample Preparation Reagent

PrepMan® Ultra Sample Preparation Reagent provides a simple way to prepare DNA from a wide range of sample types including:

- Processed foods and their ingredients
- Bacteria
- Fungi
- Mammalian tissue smears
- Hair
- Human cells (buccal swab)
- Whole blood
- Plasmid preparations

Genomic DNA that is extracted from foodborne pathogens can then be detected using the Applied Biosystems TaqMan® Pathogen Detection Kits.

Successfully Tested Bacteria, Fungi, and Food Types

Gram-positive and Gram-negative bacteria were successfully tested with PrepMan Ultra Sample Preparation Reagent as were meat, dairy, egg, and seafood samples.
Required Materials

Materials Supplied

Each bottle of PrepMan® Ultra Sample Preparation Reagent contains enough volume to extract DNA from 50 to 200 preparations.

<table>
<thead>
<tr>
<th>Item</th>
<th>Part Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrepMan® Ultra Sample Preparation Reagent</td>
<td>4322547</td>
<td>• One bottle containing 20-mL reagent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• One Protocol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• One Quick Reference Card</td>
</tr>
<tr>
<td></td>
<td>4318930</td>
<td>One bottle containing 20-mL reagent</td>
</tr>
</tbody>
</table>

Storage and Stability

Upon receipt of the PrepMan Ultra Sample Preparation Reagent, store at room temperature. Do not freeze or autoclave the PrepMan Ultra Sample Preparation Reagent.

Certificate of Analysis

The Certificate of Analysis for the PrepMan Ultra Sample Preparation Reagent can be obtained as described in the procedure under “How to Obtain Support” on page 10.

User Supplied Materials

The equipment and reagents listed below are required for sample preparation in addition to the PrepMan Ultra Sample Preparation Reagent.

Table 1 Required materials supplied by user

<table>
<thead>
<tr>
<th>Item</th>
<th>Vendor and Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment</td>
<td></td>
</tr>
<tr>
<td>Block Heater⁺</td>
<td>Major laboratory supplier (MLS)</td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Vortexer</td>
<td>MLS</td>
</tr>
<tr>
<td>Consumables</td>
<td></td>
</tr>
<tr>
<td>Aerosol-resistant pipette tips</td>
<td>MLS</td>
</tr>
<tr>
<td>Performa® DTR Gel Filtration Cartridges</td>
<td>Edge Biosystems 42453</td>
</tr>
</tbody>
</table>
Preventing Contamination

Overview

PCR assays require special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The high sensitivity of these assays can lead to amplification of a single DNA molecule (Saiki et al., 1985; Mullis and Faloona, 1987).

Preventing PCR Contamination

The following guidelines are examples and should be customized according to your laboratory setup.

- To avoid false positives due to amplified material in your work area, minimize opening tubes after amplification.
- If possible, maintain separate work areas, dedicated equipment, and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products

Table 1 Required materials supplied by user (continued)

<table>
<thead>
<tr>
<th>Item</th>
<th>Vendor and Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optional centrifuge Tube Filter (pore size 10-μm)</td>
<td>Whatman 6838-0002</td>
</tr>
<tr>
<td>Optional disposable transfer pipette</td>
<td>VWR Scientific 14670-339</td>
</tr>
<tr>
<td>Pipettor 100–1000 µL</td>
<td>MLS</td>
</tr>
<tr>
<td>Sterile microcentrifuge screw-cap tubes with attached screw-cap lid, 1.5-mL or 2-mL</td>
<td>MLS</td>
</tr>
<tr>
<td>Reagents</td>
<td></td>
</tr>
<tr>
<td>Sterile distilled water</td>
<td>MLS</td>
</tr>
</tbody>
</table>

a. The filter and the block heater are required for “Separating with a Whatman Cartridge Tube” on page 29. The block heater must accommodate 100 °C as the operating temperature.
Preventing Contamination

Note: Rooms can be simulated using a clean bench or PCR bench available from major laboratory suppliers.

- Do not bring amplified PCR products into the PCR setup area.
- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated and before you leave the work area.
- Open and close all sample tubes and reaction plates carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use positive-displacement pipettes or aerosol-resistant pipette tips.
- Clean lab benches and equipment after use with freshly diluted 10% bleach solution and/or a DNA and RNA degrading solution such as DNAZap™.
- If enough space is available, separate different targets by a row. If possible, put at least one well between unknown samples and controls.
- If possible, separate negative and positive controls by one well.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- If possible, put positive controls in one of the outer rows or columns.
- Consider that caps come in strips of 8 or 12.
- Centrifuge PCR samples briefly, whenever residual sample is present on the inside lid (for example, after dropping a tube or a plate, or when there is condensation on the tube or plate from heating or thawing).
- Before removing the caps or plate cover, briefly centrifuge the tubes or plate, or avoid cross-contamination from liquid on the caps or plate covers.

Note: If you have questions concerning PCR amplification practices, please see “How to Obtain Support” on page 10.
Prepare and Extract Samples for Bacterial and Fungal Testing

Use the following procedures to prepare and extract pathogens from your bacterial and fungal samples.

⚠️ **WARNING** **BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices).

⚠️ **WARNING** **CHEMICAL HAZARD.** PrepMan Ultra Sample Preparation Reagent contains a material that may cause eye, skin, and respiratory tract irritation, and adverse effects on the kidneys and blood and central nervous system. Please read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**IMPORTANT!** Dispose of the pipette tips and other contaminated materials in biohazard waste.

**Culture Plate Samples**

Perform the enrichment steps and PCR setup in separate areas to avoid contamination.

**Note:** Prepare a positive control tube if needed.

**To prepare bacteria and/or fungi from a culture plate:**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Shake the PrepMan Ultra Sample Preparation Reagent well, then let the reagent settle until all the bubbles have disappeared.</td>
</tr>
</tbody>
</table>
To prepare bacteria and/or fungi from a culture plate: (continued)

<table>
<thead>
<tr>
<th>Step</th>
<th>Instruction</th>
</tr>
</thead>
</table>
| 2.   | **IMPORTANT!** To prevent contamination, do not pipette directly out of the PrepMan Ultra Sample Preparation Reagent bottle into the sample tubes.  
Using 100 μL per sample and a sterile pipette, transfer the appropriate quantity of PrepMan Ultra Sample Preparation Reagent into a 50-mL sterile conical tube or other sterile container. |
| 3.   | Open the caps of all your sample tubes. |
| 4.   | Label the tubes and aseptically dispense 100 μL of the PrepMan Ultra Sample Preparation Reagent into the appropriate microcentrifuge screw-cap tube. |
| 5.   | Using an isolated colony, select a small loopful of cells or the edge of filamentous fungi colony on a culture plate.  
**Note:** The ideal colony size is 1-2 mm for bacteria and yeast and 3 mm for filamentous fungi. |
| 6.   | Suspend the cells in the100 μL of PrepMan Ultra Sample Preparation Reagent in the appropriate microcentrifuge screw-cap tube. |
| 7.   | Tightly cap the tubes, then vigorously vortex the sample for 10 to 30 seconds. |
| 8.   | Place the microcentrifuge screw-cap tubes in a heat block set to 95 °C to 100 °C for 10 minutes. To prevent contamination of the thermal cycler surface, do not heat samples in a thermal cycler. |
| 9.   | While the samples are heating, label a second set of 2-mL or other appropriate microcentrifuge screw-cap tubes. |
| 10.  | Remove the sample tubes from the heat block and allow the tubes to cool to room temperature for 2 minutes. |
| 11.  | Spin the tubes in the microcentrifuge at the highest speed for 2 minutes. |
To prepare bacteria and/or fungi from a culture plate: *(continued)*

<table>
<thead>
<tr>
<th>Step</th>
<th>Instruction</th>
</tr>
</thead>
</table>
| 12.  | Transfer 50 μL of the supernatant from the spin tubes into a second set of labeled-microcentrifuge screw-cap tubes and discard the remaining supernatant. When the supernatant is not in use, Applied Biosystems recommends storing it at 4 °C for one month or freeze them at −20 °C indefinitely. Before use, thaw, then vortex and centrifuge the stored supernatant.  
**Note:** If the sample supernatant is covered with a layer of lipid or other debris, collect clear supernatant from the center of the supernatant phase.  
**Note:** If the sample is colored or cloudy, there may be PCR inhibitors present. To help reduce the potential inhibitory effects of the sample, Applied Biosystems recommends diluting your sample minimally 1:10 before thermal cycling or following the recommendations in the protocol for your application.  
**Note:** See “Additional Procedures to Remove PCR Inhibitors” on page 32.  
DNA extraction is now complete. Refer to the assay protocol that you are using for the remaining procedures. |
| 13.  | Amplify your samples using the appropriate thermal cycling protocol. |
Prepare and Extract Samples for Bacterial and Fungal Testing

**Culture Broth Samples**

Perform the enrichment steps and PCR setup in separate areas to avoid contamination.

**Note:** Prepare a positive control tube if needed.

To prepare bacteria and/or fungi from a culture broth:

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Shake the PrepMan Ultra Sample Preparation Reagent well, then let the reagent settle until all the bubbles have disappeared.</td>
</tr>
<tr>
<td>2.</td>
<td><strong>IMPORTANT!</strong> In order to prevent contamination, do not pipet directly out of the PrepMan Ultra Sample Preparation Reagent bottle into the sample tubes. Using 100 μL per reaction and a sterile pipette, transfer the appropriate quantity of PrepMan Ultra Sample Preparation Reagent into a 50-mL sterile conical tube or other sterile container.</td>
</tr>
<tr>
<td>3.</td>
<td>Label the tubes and pipette 1 mL of culture broth containing bacteria or fungi into a new 2-mL or other appropriate microcentrifuge screw-cap tube.</td>
</tr>
<tr>
<td>4.</td>
<td>Spin the tubes in the microcentrifuge at the highest speed for 2 minutes.</td>
</tr>
</tbody>
</table>
| 5.   | Aspirate and discard the supernatant using a disposable transfer pipette.  
**Note:** Use a new pipette for each sample. Do not decant the sample.  
**IMPORTANT!** Remove as much of the supernatant as possible without disturbing the pellet.  
**IMPORTANT!** If there is a lipid layer at the top of the supernatant, draw off as much of the lipid layer as possible and discard it before removing the remainder of the supernatant with a new transfer pipette. |
| 6.   | Using a 1-mL pipette, aseptically add 100 μL of the PrepMan Ultra Sample Preparation Reagent into each tube.  
**IMPORTANT!** Change pipette tips between tubes. |
To prepare bacteria and/or fungi from a culture broth:

<table>
<thead>
<tr>
<th>Step</th>
<th>Instruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.</td>
<td>Tightly cap the tubes, then vigorously vortex the sample.</td>
</tr>
<tr>
<td>8.</td>
<td>Place the microcentrifuge screw-cap tubes in a heat block set to 100 °C for 10 minutes.</td>
</tr>
<tr>
<td>9.</td>
<td>While the samples are heating, label a second set of 2-mL or other appropriate microcentrifuge screw-cap tubes.</td>
</tr>
<tr>
<td>10.</td>
<td>Remove the sample tubes from the heat block and allow them to cool to room temperature for 2 minutes.</td>
</tr>
<tr>
<td>11.</td>
<td>Spin the tubes in the microcentrifuge at the highest speed for 2 minutes.</td>
</tr>
<tr>
<td>12.</td>
<td>Transfer 50 μL of the supernatant from the spun tubes into a second set of labeled-microcentrifuge screw-cap tubes and discard the remaining supernatant. Use 5 μL of supernatant per assay reaction. When the supernatant is not in use, Applied Biosystems recommends storing it at 4 °C for one month or freeze them at −20 °C indefinitely. Before use, thaw, then vortex and centrifuge the stored supernatant. <strong>Note:</strong> If the sample supernatant is covered with a layer of lipid or other debris, collect clear supernatant from the center of the supernatant phase. <strong>Note:</strong> If the sample is colored or cloudy, there may be PCR inhibitors present. To help reduce the potential inhibitory effects of the sample, Applied Biosystems recommends diluting your sample minimally 1:10 before thermal cycling or following the recommendations in the protocol for your application. <strong>Note:</strong> See “Additional Procedures to Remove PCR Inhibitors” on page 32.</td>
</tr>
<tr>
<td>13.</td>
<td>Amplify your samples using the appropriate thermal cycling protocol.</td>
</tr>
</tbody>
</table>
Prepare and Extract Food Samples for Pathogen Testing

Use the following procedures to prepare and extract pathogens from your food samples.

**BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices).

**CHEMICAL HAZARD.** PrepMan Ultra Sample Preparation Reagent contains a material that may cause eye, skin, and respiratory tract irritation, and adverse effects on the kidneys and blood and central nervous system. Please read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**IMPORTANT!** Dispose of the pipette tips and other contaminated materials in biohazard waste.

**Note:** Prepare a positive control tube if needed.

To prepare and extract a food test sample for pathogen testing:

1. Enrich the sample as determined by standard protocols. Follow the procedure suitable to your particular food or environmental sample type and the specific TaqMan pathogen detection assay.
   
   **Note:** For example, in a homogenizer bag, combine 25g of the food sample with 225 mL of the enrichment broth and grow overnight.
To prepare and extract a food test sample for pathogen testing: (continued)

<table>
<thead>
<tr>
<th>Step</th>
<th>Procedure</th>
</tr>
</thead>
</table>
| 2.   | Using a pipettor suction device, transfer 1 mL of enriched culture into the 2-mL or other appropriate microcentrifuge screw-cap tube.  
      **Note:** Avoid transferring food debris from the enrichment broth into the microcentrifuge screw-cap tube. |
| 3.   | Centrifuge the sample for 3 minutes at room temperature in a microcentrifuge at the highest speed to pellet bacteria and residual food or other debris. |
| 4.   | Aspirate and discard the supernatant using a disposable transfer pipette.  
      **Note:** Use a new pipette for each sample. Do not decant the sample.  
      **IMPORTANT!** Remove as much of the supernatant as possible without disturbing the pellet.  
      **IMPORTANT!** If there is a lipid layer at the top of the supernatant, draw off as much of the lipid layer as possible and discard it before removing the remainder of the supernatant with a new transfer pipette. |
| 5.   | Shake the PrepMan Ultra Sample Preparation Reagent well, then let the reagent settle until all the bubbles have disappeared before dispensing. |
| 6.   | Using 100 µL per reaction and a sterile pipette, transfer the appropriate quantity of PrepMan Ultra Sample Preparation Reagent into a 50-mL conical tube or other sterile container.  
      **IMPORTANT!** In order to prevent contamination, do not pipet directly out of the PrepMan Ultra Sample Preparation Reagent bottle into the sample tubes. |
| 7.   | Open the caps of all your sample tubes. |
To prepare and extract a food test sample for pathogen testing:
(continued)

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
</table>
| 8.   | Label the tubes, then using a 1-mL pipette, aseptically add 100 µL of the PrepMan Ultra Sample Preparation Reagent into the tube containing the sample.  
  **IMPORTANT!** Change pipette tips between tubes. |
| 9.   | Tightly cap the tubes, then vigorously vortex to resuspend the pellet. |
| 10.  | Place the microcentrifuge screw-cap tubes in a heat block set to 100 °C for 10 minutes. |
| 11.  | While the samples are heating, label a second set of 2-mL or other appropriate microcentrifuge screw-cap tubes. |
| 12.  | Remove the sample tubes from the heat block and allow them to cool to room temperature for 2 minutes. |
| 13.  | Spin the tubes in the microcentrifuge at 12,000 rpm for 2 minutes. |
To prepare and extract a food test sample for pathogen testing: (continued)

<table>
<thead>
<tr>
<th>Step</th>
<th>Protocol Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.</td>
<td>Transfer 50 μL of the supernatant from the spun tubes into a second set of labeled-microcentrifuge screw-cap tubes and discard the remaining supernatant. Use 5 μL of supernatant per assay reaction. This procedure requires a Performa DTR Gel Filtration Cartridge from Edge Biosystems (see “User Supplied Materials” on page 13). When the supernatant is not in use, Applied Biosystems recommends storing it at 4 °C for one month or freeze them at −20 °C indefinitely. Before use, thaw, then vortex and centrifuge the stored supernatant. <strong>Note:</strong> If the sample supernatant is covered with a layer of lipid or other debris, collect clear supernatant from the center of the supernatant phase. <strong>Note:</strong> If the sample is colored or cloudy, there may be PCR inhibitors present. To help reduce the potential inhibitory effects of the sample, Applied Biosystems recommends diluting your sample minimally 1:10 before thermal cycling or following the recommendations in the protocol for your application. <strong>Note:</strong> See “Additional Procedures to Remove PCR Inhibitors” on page 32.</td>
</tr>
<tr>
<td>15.</td>
<td>Amplify your samples using the appropriate thermal cycling protocol.</td>
</tr>
</tbody>
</table>
Prepare and Extract Food Samples for GMO Testing

The following procedure can be used with TaqMan® Genetically Modified Organism (GMO) Detection Kits (soy kit PN 4327692; maize kit PN 4327693).

The food sample must first be crushed or chopped into very fine grains. A powdered sample such as soy flour does not need processing, but solid samples such as whole soybean, whole maize kernels, and solid foods, do need this processing.

Certified standard reference materials that are made from genetically modified soy or maize, which are available from the Institute for Reference Materials and Measurements (IRMM), are supplied in powdered form and need no additional processing.

To prepare and extract a food test sample:

1. Weigh 20 mg of each sample or concentration reference standard into a 2-mL or other appropriate screw-cap microcentrifuge screw-cap tube.

2. Shake the PrepMan Ultra Sample Preparation Reagent well, then let the reagent settle until all the bubbles have disappeared.

3. Using 400 µL per reaction and a sterile pipette, transfer the appropriate quantity of PrepMan Ultra Sample Preparation Reagent into a 50-mL conical tube or other sterile container.

   **IMPORTANT!** In order to prevent contamination, do not pipet directly out of the PrepMan Ultra Sample Preparation Reagent bottle into the sample tubes.

4. Open the caps of all your sample tubes.

5. Using a 1-mL pipette, aseptically add 400 µL of PrepMan Ultra Sample Preparation Reagent to each 20 mg sample.

   **IMPORTANT!** Change pipette tips between tubes.
To prepare and extract a food test sample: *(continued)*

<table>
<thead>
<tr>
<th>Step</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>Tightly cap the tubes, then vigorously vortex to resuspend the pellet.</td>
</tr>
<tr>
<td>7.</td>
<td>Place the microcentrifuge screw-cap tubes in a heat block set to 100 °C for 10 minutes.</td>
</tr>
<tr>
<td>8.</td>
<td>While the samples are heating, label a second set of 2-mL or other appropriate microcentrifuge screw-cap tubes.</td>
</tr>
<tr>
<td>9.</td>
<td>Remove the sample tubes from the heat block and allow them to cool to room temperature for 2 minutes.</td>
</tr>
<tr>
<td>10.</td>
<td>Spin the tubes in the microcentrifuge at 12,000 rpm for 2 minutes.</td>
</tr>
</tbody>
</table>
| 11.  | Transfer 50 μL of the supernatant from the spun tubes into a second set of labeled-microcentrifuge screw-cap tubes and discard the remaining supernatant. Use 5 μL of supernatant per assay reaction.  

When the supernatant is not in use, Applied Biosystems recommends storing it at 4 °C for one month or freeze them at −20 °C indefinitely. Before use, thaw, then vortex and centrifuge the stored supernatant.  

**Note:** If the sample supernatant is covered with a layer of lipid or other debris, collect clear supernatant from the center of the supernatant phase.  

**Note:** If the sample is colored or cloudy, there may be PCR inhibitors present. To help reduce the potential inhibitory effects of the sample, Applied Biosystems recommends diluting your sample minimally 1:10 before thermal cycling or following the recommendations in the protocol for your application.  

**Note:** See “Additional Procedures to Remove PCR Inhibitors” on page 32. |
| 12.  | Amplify your samples using the appropriate thermal cycling protocol. |
Appendix A - Filtration Methods

Filtration Methods for Separating Fine Particulates (Optional)

About Filtering Samples with Fine Particulates

If your sample contains fine particulates, you may select from two alternative procedures:

- “Separating with a Disposable Funnel” below, or
- “Separating with a Whatman Cartridge Tube” on page 29

Note that your choice of procedure determines whether or not you follow all or part of “Prepare and Extract Samples for Bacterial and Fungal Testing” on page 16.

Select one of the two procedures provided below to separate fine, abundant, particulate (for example, cocoa, spices, or juice precipitates) from the enrichment medium.

**WARNING** BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices).

Separating with a Disposable Funnel

To separate using a disposable funnel:

1. Mount a disposable funnel lined with a paper filter above a 15-mL disposable reagent tube.

2. Transfer approximately 10 mL of post-enrichment material into the filter and collect at least 2 mL of filtrate.

**IMPORTANT!** Dispose of the filter and funnel in a biohazard waste receptacle.
Appendix A - Filtration Methods

Separating with a Whatman Cartridge Tube

To separate using a disposable funnel: (continued)

3. Transfer 1 mL of the collected filtrate into a 2-mL or other appropriate microcentrifuge screw-cap tube.

4. Proceed to steps 1 and 2, then 4 through 13 of “Culture Broth Samples” on page 19.

WARNING CHEMICAL HAZARD. PrepMan Ultra Sample Preparation Reagent contains a material that may cause eye, skin, and respiratory tract irritation, and adverse effects on the kidneys and blood and central nervous system. Please read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: Step 1 of the following procedure continues the enrichment process.

To prepare a sample by Whatman centrifuge tube filtration:

1. Prepare the sample for filtration.
   a. Remove the homogenizer bag from the incubator.
      IMPORTANT! Make sure that the bag is tightly sealed to avoid spillage of biohazardous material during rocking.
   b. Rock the bag from side-to-side to mix the contents of the bag.
   c. Let the contents of the homogenizer bag stand on the bench top for at least 10 minutes while the debris settles to the bottom of the bag.

2. With the filter insert in the outer tube (supplied assembled), pipette approximately 0.5 mL of enriched material into the Whatman tube filter.
   IMPORTANT! Do not touch membrane surface with the tip.
   IMPORTANT! Dispose of pipette tips in biohazard waste.

3. Seal the tube using the tethered cap.
4. Centrifuge the tube.
   a. Place the tube into a microcentrifuge, ensuring that the centrifuge is evenly balanced. Do not invert or tilt the tube.
   b. Centrifuge the sample for 3 minutes at the highest speed, then open the cap and remove the filter insert.
      **Note:** You may spin the tube for a longer time to ensure that most of the fluid has penetrated through the insert.
   c. Dispose of the filter insert in a biohazard waste receptacle.

5. Using a disposable transfer pipette, carefully aspirate and discard the supernatant without disturbing the pellet at the bottom of the tube.
   **IMPORTANT!** Dispose of the pipette and supernatant in a biohazard waste receptacle.

6. Transfer 100 µL of the PrepMan Ultra Sample Preparation Reagent into the tube containing the bacterial pellet.

7. Resuspend the pellet homogeneously by pipetting up and down.

8. Place the tube in a preheated block heater and heat it at 100 °C for 10 minutes, then remove it from the heater and allow the tube to cool for 2 minutes.

9. Centrifuge the tube in the microcentrifuge at the highest speed for 3 minutes.
To prepare a sample by Whatman centrifuge tube filtration: (continued)

<table>
<thead>
<tr>
<th>Step</th>
<th>Instruction</th>
</tr>
</thead>
</table>
| 10.  | Transfer 50 μL of the supernatant from the spun tube into a second labeled microcentrifuge screw-cap tube and discard the remaining supernatant. Your samples should be clear and colorless.  
**Note:** If the sample is colored or cloudy, there may be PCR inhibitors present. Dilute the sample at minimally 1:10 dilution using sterile, distilled water before proceeding to thermal cycling. |
| 11.  | Amplify your samples using the appropriate thermal cycling protocol. |

**Sedimentation Method for Separating Gross Particulates (Optional)**

If an enrichment sample contains gross particulates (such as ground meat), and the homogenizer bag does not have a mesh filter layer, let the contents of the homogenizer bag stand on the bench top for 5 minutes while the debris settles to the bottom of the bag. Then proceed to step 5 of the Prepare and Extract Food Samples for Pathogen Testing procedure on page 21.

⚠️ **WARNING:** BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices).
Appendix B - Troubleshooting

Table 2  Troubleshooting the PrepMan Ultra Sample Reagent process

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PCR amplification of sample</td>
<td>Presence of PCR inhibitors or fluorescent contaminants</td>
<td>Remove PCR inhibitors or contaminants by following one of the procedures listed here:</td>
</tr>
<tr>
<td>Sample is cloudy or contains color</td>
<td></td>
<td>• “Additional Procedures to Remove PCR Inhibitors” below</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• “Precipitating Nucleic Acids” on page 33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• “Performing Spin Column Purification” on page 34</td>
</tr>
<tr>
<td>Inconsistent results</td>
<td>Not enough PrepMan Ultra Sample Preparation Reagent used</td>
<td>Redo analysis using correct amount of PrepMan Ultra Sample Preparation Reagent.</td>
</tr>
<tr>
<td>Error in sample preparation</td>
<td>Incorrect thermal cycling parameters</td>
<td>Refer to the thermal cycling protocol for the appropriate assay protocol.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A gel-like precipitate in the PrepMan Ultra Sample Preparation Reagent</td>
<td>Inappropriate storage or handling temperature for reagent</td>
<td>Continued usage of this reagent may lead to inconsistent results. Stop using the current batch and obtain new reagent.</td>
</tr>
</tbody>
</table>

Additional Procedures to Remove PCR Inhibitors

PCR inhibitors present in the template extract can be removed from the sample by:

- “Serially Diluting the Sample” on page 33
- “Precipitating Nucleic Acids” on page 33
- “Performing Spin Column Purification” on page 34

Note: The sample used in these procedures should have been prepared in step 12 of the “Culture Plate Samples” procedure on page 16.
Serially Diluting the Sample

PCR inhibitors in the sample can be removed through dilution. The drawback of diluting the sample is that the actual target may be removed if it is present in low numbers.

**Note:** Applied Biosystems recommends setting up small, incremental dilutions rather than initially diluting the sample in a large volume.

Prepare 10-fold serial dilutions of 1:10, 1:100, and 1:1000. Do not exceed 1:1000.

Precipitating Nucleic Acids

Nucleic acid precipitation is an alternative method for removing PCR inhibitors.

**CHEMICAL HAZARD.** 3 M Sodium Acetate causes eye, skin, and respiratory tract irritation. **Isopropanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. Exposure may cause central nervous system effects such as drowsiness, dizziness, and headache. Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**To precipitate nucleic acids:**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Transfer 50 μL of the sample extract into a fresh 1.5-mL microcentrifuge screw-cap tube.</td>
</tr>
<tr>
<td>2.</td>
<td>Add 400 μL of TE buffer.</td>
</tr>
<tr>
<td>3.</td>
<td>Add 50 μL of 3 M sodium acetate and vortex the tube.</td>
</tr>
<tr>
<td>4.</td>
<td>Add 500 μL of isopropanol and vortex the tube.</td>
</tr>
<tr>
<td>5.</td>
<td>Let the sample stand at room temperature for at least 15 minutes.</td>
</tr>
<tr>
<td>6.</td>
<td>Pellet the sample by spinning in a microcentrifuge at 13,000 × g for 10 minutes at room temperature.</td>
</tr>
<tr>
<td>7.</td>
<td>Decant the supernatant without disturbing the pellet and allow the pellet to air dry.</td>
</tr>
<tr>
<td>8.</td>
<td>Resuspend the pellet in 50 μL of sterile distilled water.</td>
</tr>
</tbody>
</table>
Performing Spin Column Purification

Spin column purification is an alternative method for removing PCR inhibitors. This procedure requires a Performa DTR Gel Filtration Cartridge from Edge Biosystems (see “User Supplied Materials” on page 13) and a slow-speed microcentrifuge.

To perform spin column purification:

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pack the column by placing the unit (cartridge and microtube) in a microcentrifuge and centrifuge at $750 \times g$ for 2 minutes.</td>
</tr>
<tr>
<td>2.</td>
<td>Transfer the cartridge to a clean labeled microcentrifuge screw-cap tube.</td>
</tr>
<tr>
<td>3.</td>
<td>Add 50 μL of the sample to the packed column. Make sure that fluid runs into the gel.</td>
</tr>
<tr>
<td>4.</td>
<td>Close the cap and centrifuge for 2 minutes at $750 \times g$.</td>
</tr>
<tr>
<td>5.</td>
<td>Retain the eluate, and remove and discard the cartridge.</td>
</tr>
<tr>
<td>6.</td>
<td>Use 5 μL of the eluate for PCR analysis.</td>
</tr>
</tbody>
</table>

To precipitate nucleic acids: (continued)

9. Use 5 μL of the sample for PCR analysis.
References

Center for Disease Control and Prevention and National Institutes of Health, Biosafety in Microbiological and Biomedical Laboratories 1993. Publication Number (CDC) 93-8395.


