PrepMan™ Ultra

Sample Preparation Reagent

Protocol

For Research Use Only.
Not for use in diagnostic procedures.

Applied Biosystems

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Introduction

Purpose of PrepMan Ultra Sample Preparation Reagent

PrepMan™ Ultra Sample Preparation Reagent provides a simple way to prepare DNA from processed food and their ingredients, bacteria and fungi and food enriched homogenates that may contain Gram negative pathogens. Genomic DNA that is extracted from the food-borne pathogens may then be detected using a TaqMan® nuclease detection assay.

Materials Supplied

Kit Contents

The PrepMan Ultra Sample Preparation Reagent contains enough volume to extract DNA from 50 to 100 preparations.

Item	Part Number	Description
PrepMan Ultra Sample Preparation Reagent	4322547	♦ One 30-mL bottle ♦ One Protocol ♦ One Quick Start Card
	4318930	One 30 mL bottle containing 20 mL of reagent
Quick Reference Card	4318924	Summarizes protocol procedures
Protocol	4318925	Provides procedures for using PrepMan Ultra Sample Preparation Reagent

Storage and Stability

Upon receipt of the PrepMan Ultra Sample Preparation Reagent, store at 21–23 °C.

⚠ CAUTION Do not freeze or autoclave the PrepMan Ultra Sample Preparation Rreagent.

Certificate of Analysis

The Certificate of Analysis for PrepMan Ultra Sample Preparation Reagent can be obtained as described in the procedure under “To Obtain Technical Documents” on page 30.

Materials Supplied by the User for Sample Preparation

User Supplied Materials

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

Item	Vendor
Aerosol-resistant pipet tips	Major laboratory supplier (MLS)
Block Heater[a]	MLS
Boiling water bath	MLS
Centriflex Gel filtration Cartridgel[b]	Edge Biosystems (P/N 42453)
Centrifuge Tube Filter[a] (pore size 10-µm)	Whatman (P/N 6838-0002)
Disposable reagent tube, 50-mL	MLS
Disposable transfer pipet	VWR Scientific (P/N 14670-339)
Floating 2-mL tube holder or rack	MLS
Isopropanol[c]	MLS
Microcentrifuge	MLS
Paper filter[d]	Schleicher and Schuell, Inc.
Pre-enrichment broth	MLS
Pipettor 100–1000 µL	MLS
Seward Stomacher	MLS
3M sodium acetate[c]	MLS
Sterile distilled water	MLS
Sterile microcentrifuge tubes with attached screw-cap lid, 2-mL	MLS
TE buffer[c]	MLS
Vortexer	MLS
Whirl-Pak® filter bags for Stomacher	Nasco West (P/N B01318WA)

a. The filter and the block heater are required for performing the “Whatman Cartridge Tube Procedure” on page 16.

b. This cartridge is required for performing “Spin Column Purification” on page 20.

c. TE buffer, 3M sodium acetate, and isopropanol are required for performing “Nucleic Acid Precipitation” on page 20.

d. The paper filter is required for performing “Alternative Procedure B - Filtration Method” on page 16.

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, 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 which, if not avoided, could result in death or serious injury.

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

General Biohazard Warning

⚠ WARNING **BIOHAZARD**. Biological samples such as tissues and blood have the potential to transmit infectious diseases. Follow the U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4) and in Occupational Safety and Health Standards, Toxic and Hazardous Substances (29 CFR §1910.1030) concerning the principles of risk assessment, biological containment, and safe laboratory practices for activities involving clinical specimens. You can obtain additional information by connecting to the government Web site http://www.cdc.gov.

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.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems kits are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- 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.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

To order documents by automated telephone service:

1	From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2	Follow the voice instructions to order documents (for delivery by fax). **Note** There is a limit of five documents per fax request.

To order documents by telephone:

In the U.S.	Dial **1.800.345.5224**, and press **1**.
In Canada	♦ To order in English, dial **1.800.668.6913** and press **1**, then **2**, then **1** ♦ To order in French, dial **1.800.668.6913** and press **2**, then **2**, then **1**
From any other country	See the specific region under “To Contact Technical Support by Telephone or Fax (Outside North America)” .

To view, download, or order documents through the Applied Biosystems web site:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, click **Documents on Demand**, then click **MSDS.**
3	Click **MSDS Index**, search through the list for the chemical of interest to you, then click on the MSDS document number for that chemical to open a pdf of the MSDS.

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

Recommended PCR Amplification Practices

General PCR Practices

The DNA amplification capability of the PCR process makes special laboratory practices necessary. The following precautions will minimize sample cross-contamination and PCR product carryover (Kwok and Higuchi, 1989):

- Wear a clean lab coat and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Use air-displacement or positive-displacement pipettors with filter-plugged tips. Change tips after each use.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution.

Note If you have questions concerning PCR amplification practices, please see “Technical Support” on page 23 for phone numbers.

Sample Preparation for Processed Food Samples

Introduction

The following method can be used with TaqMan® Genetically Modified Organism (GMO) Detection Kits (the soy kit is P/N 4327692 and the maize kit is P/N 4327693).

The food sample must first be crushed or chopped into very fine grains. A powdered sample such as soy flour needs no such processing, but solid samples such as whole soybean, whole maize kernels, solid foods, etc. need this processing. Certified reference materials such as the genetically modified soy or genetically modified maize concentration standards produced by the Institute for Reference Materials and Measurements (IRMM) are supplied in powdered form and need no additional processing.

Extraction Procedure

To prepare processed food samples:

Step	Action
1	Weigh 20 mg of each sample and concentration reference standard into a 2-mL screw-cap microcentrifuge tube.
2	Add 400 µL of PrepMan™ Ultra Sample Preparation Reagent to each 20 mg sample. **Note** For instructions on dispensing PrepMan Ultra Sample Preparation Reagent, see page 14. **⚠ WARNING CHEMICAL HAZARD**. **PrepMan Ultra** 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 MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Vortex the sample to suspend the food sample completely.
4	Heat the sample for 10 min at 100 °C in a water bath.
5	Centrifuge the sample for 2 min at high speed in a microcentrifuge.
6	Remove the cleared supernatant from each prep and place it in a fresh microcentrifuge tube. **Note** Samples may have a slight color, and there may be slight turbidity which forms immediately or with storage. This in normal, and no additional steps need to be taken.
7	Store the supernatants frozen at –20 °C indefinitely.
8	Use 5 µL of supernatant per assay reaction.

Sample Preparation for Bacterial and Fungal Cultures

Introduction

Prepare your sample using the procedure provided in this section.

⚠ WARNING Observe Biosafety Level 2 practices (CDC, 1993), containment equipment, and facilities for working with cultures known to contain or potentially containing microbial pathogens.

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

Extraction Procedure

To prepare a sample:

Step	Action
1	Follow either step a or step b, depending upon the source of your sample. a. From a culture broth containing bacteria or fungi: – Pipet 1 mL of culture broth containing bacteria or fungi into a new 2.0-mL microcentrifuge tube. **Note** 500 µL tubes are also suitable if you are using a thermocycler or block heater as a heating source. – Spin the sample for 3 min at 16,000 x *g*. – Add 200 µL of PrepMan Ultra Sample Preparation Reagent. Close the cap tightly. **Note** For instructions on dispensing PrepMan Ultra Sample Preparation Reagent, see page 14. b. From a culture plate: – Select a small loopful of cells or edge of filamentous fungi colony from the culture plate. – Suspend the cells in 200 µL of PrepMan Ultra Sample Preparation Reagent in a 2.0-mL microcentrifuge tube. **Note** 500 µL tubes are also suitable if you are using a thermocycler or block heater as a heating source. **Note** For instructions on dispensing PrepMan Ultra Sample Preparation Reagent, see page 14. **⚠ WARNING CHEMICAL HAZARD**. **PrepMan Ultra** 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 MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Vortex the sample for 10 to 30 sec (until the cells are homogeneously suspended in reagent.)
3	Heat the sample for 10 min at 100 °C in a water bath.
4	Spin the sample for 3 min at 16,000 x *g*.
5	Transfer the supernatant to a new microcentrifuge tube, The supernatant is ready for PCR.

To prepare a sample: *(continued)*

Step	Action
6	For PCR, use an appropriate quantity of supernatant for your assay. For example: ♦ Use 1 µL directly from the supernatant for the MicrSeq® 500 16S r DNA Bacterial Sequencing Kit or the MicroSeq™ D2 LSU rDNA Fungal Sequencing Kit. ♦ Use 5 µL directly from the supernatant for the TaqMan™ pathogen detection assay. **Note** If you have a very concentrated culture (>10^8 cfu/mL) to start with, use 1:10 diluent (in sterile deionized water) instead of direct lysate from step 5 to prepare for PCR.

Sample Preparation for Pathogens from Food Samples

Introduction

The following procedures are provided for sample preparation of pathogens from food supplies:

- About About Enriching the Sample - see below
- General Extraction Procedures - see page 13
  - Preparing a Test Sample
  - Dispensing the PrepMan Ultra Sample Preparation Reagent
  - Boiling and Diluting the Sample
  - Thermal Cycling
- Alternative Procedure A - Sedimentation Method - see page 15
- Alternative Procedure B - Filtration Method - see page 16

About Enriching the Sample

The enrichment step allows time for any viable food-borne microorganisms to proliferate. Thus, viable organisms are present in levels detectable by TaqMan® pathogen assay analysis.

⚠ WARNING Observe Biosafety Level 2 practices (CDC, 1993) containment equipment, and facilities for working with cultures known to contain or potentially containing microbial pathogens.

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

Homogenizer Blender Bags with Filter

While enriching the sample, we recommend the use of Whirl-Pak bags with a filter layer. These bags contain a layer of fine polyethylene mesh that divides the inside of the bag into two sections.

For example, if a meat sample is placed on one side of the mesh filter layer for pre-enrichment, the majority of fat and gross particulate will remain on that side of the mesh filter layer. The mesh separates solid food from the enriched medium and enables the user to remove a sample of the enrichment medium without pipetting gross particulate and fat.

Enriching the Samples

To enrich the samples:

Step	Action
1	Enrich the sample as determined by standard protocols. Follow the procedure suitable to the particular food or environmental sample type. **Note** For example, in a homogenizer bag combine 25 g of the food sample with 225 mL of the enrichment broth recommended for your specific TaqMan pathogen detection assay.
2	Gently homogenize in a Stomacher instrument between 30 sec and 2 min.
3	Incubate at 35–37 °C for the length of time recommended for your specific TaqMan pathogen detection assay.
4	Label one sterile 2-mL microcentrifuge tube with attached screw cap for each sample. It is most effective to label the tubes on the top of the screw cap. **Note** Microcentrifuge tubes with attached screw cap lids are recommended in order to avoid the following: – Lids opening during sample boiling – Sample cross-contamination (resulting from lids being placed on the wrong sample tube)
5	If the enrichment sample contains: ♦ Gross particulate, and the homogenizer bag has a mesh filter layer, then proceed to “General Extraction Procedures” on page 13. ♦ Gross particulate, and the homogenizer bag does not have a mesh filter layer, then proceed to the “Alternative Procedure A - Sedimentation Method” on page 15. ♦ Fine particulate (*e.g.*, cocoa powder) or chocolate, or is rich in pigment such as herbs and spices, then proceed to “Alternative Procedure B - Filtration Method” on page 16.

General Extraction Procedures

The following procedures are used for extraction:

- Preparing a Test Sample - see below
- Dispensing the PrepMan Ultra Sample Preparation Reagent - see page 14
- Boiling and Diluting the Sample - see page 14
- Thermal Cycling - see page 15

Preparing a Test Sample

Step	Action
1	Using a 1-mL pipet tip and pipettor, transfer 1 mL of enrichment broth into the 2-mL microcentrifuge tube. **⚠ WARNING** Observe Biosafety Level 2 practice (CDC,1993) containment equipment, and facilities for working with cultures known to contain or potentially containing microbial pathogens. **Note** Avoid transferring food debris from the enrichment broth into the microcentrifuge tube. **⚠ WARNING** Dispose of pipet tips in biohazard waste.
2	If analyzing a food sample for the first time, a preparation of a positive control tube is recommended. This is prepared by spiking 1 x 10^6 colony forming units (cfu) of the food pathogen into 1 mL of enrichment broth.
3	Spin the sample for 3 min at room temperature in a microcentrifuge at maximum speed (16,000 x *g*). This will pellet bacteria and residual food or other debris.
4	Aspirate and discard the supernatant using a disposable transfer pipet. ♦ Use a new pipet 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, be sure to draw off as much of the lipid layer as possible and discard before removing the remainder of the supernatant with a new transfer pipet. **⚠ WARNING** Dispose of the pipet tips and supernatant in biohazard waste.

Dispensing the PrepMan Ultra Sample Preparation Reagent

To prepare and dispense:

Step	Action
1	Shake well before dispensing the PrepMan Ultra Sample Preparation Reagent. Let the reagent settle until all the bubbles have disappeared. **⚠ 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 MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	In order to prevent contamination, do not pipet directly out of the PrepMan Ultra Sample Preparation Reagent bottle into the sample tubes. Instead, transfer the appropriate amount using a sterile pipet (200 µL per reaction is required). Pipet into a clear 50-mL conical tube or other sterile container.
3	Open the caps of all the tubes.
4	Using a 1-mL pipet, aseptically transfer 200 µL of the PrepMan Ultra Sample Preparation Reagent into the 2-mL screw-cap microcentrifuge tube containing the bacterial pellet. **IMPORTANT** Change pipet tips between tubes.
5	Tightly cap the tubes and resuspend the pellet by vigorously mixing the tube using a vortex mixer until the pellet is resuspended.

Boiling and Diluting the Sample

To boil and dilute the sample:

Step	Action
1	Place the microcentrifuge tubes in a floating tube holder or rack and place in a boiling water bath for 10 min. **Note** While the samples are boiling, label a second set of 2-mL microcentrifuge tubes (one for each sample).
2	Remove the sample tubes from the boiling water and allow the tubes to cool to room temperature for 2 min.
3	Spin the tubes in the microcentrifuge at 16,000 x *g* for 3 min.

To boil and dilute the sample: *(continued)*

Step	Action
4	a. Transfer 50 µL of the supernatant from the spun tubes into a second set of labeled-microcentrifuge tubes. b. Discard the remaining supernatant. **Note** If the sample supernatant is covered with a layer of lipid or other debris, try to collect clear supernatant from the center of the supernatant phase.
5	The above sample should appear colorless. If color or cloudiness is present in the sample, there may be PCR inhibitors present. ♦ If the sample is cloudy or has color, it is necessary to dilute the sample. Dilute the sample 1:2 or 1:5 using sterile distilled water. See "Additional Procedures to Remove PCR Inhibitors" on page 19. ♦ If the sample is clear and colorless, proceed to the next step. **Note** This tube contains the sample used for the TaqMan pathogen detection assay.
6	PCR amplify this sample. The sample can be stored at 4 °C for up to one month.

Thermal Cycling

Use the appropriate thermal cycling profile as described in the kit protocol.

Alternative Procedure A - Sedimentation Method

Follow the procedure below if the enrichment sample contains gross particulate (*e.g.,* ground meat), and the homogenizer bag does not have a mesh filter layer.

⚠ WARNING Observe Biosafety Level 2 practices (CDC, 1993) containment equipment, and facilities for working with cultures known to contain or potentially containing microbial pathogens.

To prepare a sample with gross particulate:

Step	Action
1	Let the contents of the homogenizer bag stand on the bench top for 5 min while the debris settles to the bottom of the bag.
2	Proceed to "Dispensing the PrepMan Ultra Sample Preparation Reagent" on page 14.

Alternative Procedure B - Filtration Method

Follow one of the two procedures provided below to separate fine, abundant, particulate (*e.g.,* cocoa, spices, juice precipitates) from the enrichment medium. The first procedure uses a disposable funnel and the second procedure uses a Whatman® Centrifuge tube filter.

⚠ WARNING Observe Biosafety Level 2 practices (CDC, 1993) containment equipment, and facilities for working with cultures known to contain or potentially containing microbial pathogens.

Disposable Funnel Procedure

To separate using a disposable funnel:

Step	Action
1	Mount a disposable funnel lined with a paper filter above a 15-mL disposable reagent tube.
2	Transfer approximately 10 mL of pre-enrichment material into the filter and collect at least 2 mL of filtrate. **⚠ WARNING** Dispose of the filter and funnel in biohazard waste receptacle.
3	Transfer 1 mL of the collected filtrate into a 2-mL microcentrifuge tube.
4	Proceed to step 3 of the procedure under “Preparing a Test Sample” on page 13.

Whatman Cartridge Tube Procedure

Note Step 1 of the procedure below continues the enrichment process.

To prepare a sample by Whatman centrifuge tube filtration:

Step	Action
1	Do the following to prepare for using the Whatman Cartridge tube filter. a. Remove the homogenizer bag from the incubator. **⚠ WARNING** Before rocking, make sure the bag is tightly sealed to avoid spillage of biohazardous material. 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 min while the debris settles to the bottom of the bag.

To prepare a sample by Whatman centrifuge tube filtration: *(continued)*

Step	Action
2	With the filter insert in the outer tube (supplied assembled), pipet approximately 0.5 mL of enrichment material into the Whatman tube filter. **⚠ WARNING** Dispose of pipet tips in biohazard waste. **IMPORTANT** If using a pipet or pipettor, do not touch membrane surface with tip.
3	Seal the tube using the tethered cap and centrifuge as follows: 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 min at 16,000 *x g*, then open the cap and remove the filter insert. (Spin for a longer time to ensure that most of the fluid has penetrated through the insert.) c. Dispose of the filter insert. **⚠ WARNING** Dispose of the filter insert in biohazard waste receptacle.
4	Aspirate and discard the supernatant, without disturbing the pellet at the bottom of the tube, using a disposable transfer pipet. **⚠ WARNING** Dispose of the pipet and supernatant in biohazard waste receptacle.
5	Prepare the sample and heat as follows: a. Transfer 100 µL of the PrepMan Ultra Sample Preparation Reagent into the tube containing the bacterial pellet. **⚠ 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 MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. b. Resuspend the pellet homogeneously by pipetting. c. Place the tube in a preheated block heater and heat at 100 °C for 10 min.
6	a. Turn off the heater and allow the tube to cool down for 2 min. b. Transfer the tube to a microcentrifuge.
7	Spin the tube in the microcentrifuge at 16,000 *x g* for 3 min.

To prepare a sample by Whatman centrifuge tube filtration: *(continued)*

Step	Action
8	a. Transfer 50 µL of the supernatant from the spun tube into a second labeled microcentrifuge tube. b. Discard the remaining supernatant.
9	Take one of the following actions (depending upon the condition of the sample: ♦ If the sample is cloudy or has color, dilute the sample 1:2 or 1:5 using sterile distilled water. ♦ If the sample is clear and colorless, proceed to the next step.
10	PCR amplify this sample. The sample can be stored at 4 °C for up to one month.

Additional Procedures to Remove PCR Inhibitors

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

- Serial Dilution - see below
- Nucleic Acid Precipitation - see page 20
- Spin Column Purification - see page 20

IMPORTANT The sample used in these procedures should have been prepared through step 5 of "Boiling and Diluting the Sample" on page 15.

Serial Dilution

PCR inhibitors in the sample can be easily diluted out in water. The drawback of diluting the sample is that the actual target may be diluted out if it is present in low numbers.

IMPORTANT Setting up small, incremental dilutions is recommended rather than initially diluting the sample in a large volume.

To serially dilute the sample:

Step	Action
1	Label five 1.5-mL microcentrifuge tubes as follows: 1, 2, 3, 4, 5
2	Add 50 µL of sterile distilled water to each tube.
3	a. Pipet 50 µL of the sample into tube 1. b. Vortex. **Note** This is a 1:2 dilution of the original template extract.
4	a. Using a fresh pipet tip, transfer 50 µL from tube 1 into tube 2. b. Vortex. **Note** This is a 1:4 dilution of the original template extract.
5	a. Using a fresh pipet tip, transfer 50 µL from tube 2 into tube 3. b. Vortex. **Note** This is a 1:8 dilution of the original template extract.
6	Repeat this process to make a 1:16 and 1:32 dilution of the original template extract.
7	Retest the diluted samples in tubes 1–5. **Note** For most food types, PCR inhibitory substances will be diluted out in tubes 4 or 5.

Nucleic Acid Precipitation

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

To precipitate nucleic acids:

Step	Action
1	Transfer 50 µL of the sample extract into a fresh 1.5-mL microcentrifuge tube.
2	Add 400 µL of TE buffer (10 mM Tris, 1 mM EDTA).
3	a. Add 50 µL of 3 M sodium acetate. b. Vortex. **⚠ CAUTION** **CHEMICAL HAZARD**. **3M sodium acetate** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4	a. Add 500 µL of isopropanol. b. Vortex. **⚠ WARNING** **CHEMICAL HAZARD**. **Isopropanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache, etc. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
5	Let the sample stand at room temperature for at least 15 min.
6	Pellet the sample by spinning in a microcentrifuge at 13,000 x *g* for 10 min at room temperature.
7	Decant the supernatant without disturbing the pellet and allow the pellet to air dry.
8	Resuspend the pellet in 50 µL of sterile distilled water.
9	Use 5 µL of the sample for PCR analysis.

Spin Column Purification

Spin column purification is an alternative method for removing PCR inhibitors. This procedure requires a Centriflex Gel filtration Cartridge from Edge Biosystems (see page 2) and a slow-speed microcentrifuge.

Edge Biosystems can be reached by phone at 1-800-326-2685 and online at http://www.edgebio.com.

To perform spin column purification:

Step	Action
1	a. Pack the column by placing the unit (cartridge and microtube) in a microcentrifuge. b. Spin at 750 x *g* for 2 min.
2	a. Transfer the cartridge to a clean labeled microcentrifuge tube. b. Add 50 µL of the sample to the packed column. Make sure that fluid runs into the gel.
3	Close the cap and spin for 2 min at 750 x *g*.
4	Remove and discard the cartridge while retaining the eluate.
5	Use 5 µL of the eluate for PCR analysis.

Troubleshooting

Troubleshooting Table

Observation	Possible Cause	Action
No PCR amplification of sample Sample is cloudy or contains color	Presence of PCR inhibitors or fluorescent contaminants	Remove PCR inhibitors or contaminants by following one of the procedures listed here: ♦ "Additional Procedures to Remove PCR Inhibitors" on page 19 ♦ "Nucleic Acid Precipitation" on page 20 ♦ "Spin Column Purification" on page 20
Inconsistent results	Not enough PrepMan Ultra Sample Preparation Reagent used	Redo analysis using correct amount of PrepMan Ultra Sample Preparation Reagent.
	Error in sample preparation	
	Incorrect thermal cycling parameters	Refer to the thermal cycling protocol for the appropriate TaqMan assay protocol.
A gel-like precipitate in PrepMan Ultra Sample Preparation Reagent	Inappropriate storage or handling temperature for reagent	Continued usage of this reagent may lead to inconsistent results. Stop using the current batch and obtain new reagent.

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support:

- By e-mail
- By telephone or fax
- Through the Applied Biosystems web site

You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems web site. (Please see the section “To Obtain Technical Documents” following the telephone information below).

To Contact Technical Support by E-Mail

You can contact Applied Biosystems Technical Support by e-mail for help in the following product areas:

Product/Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems (Real-Time PCR) and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide, and DNA Synthesis	corelab@appliedbiosystems.com

Product/Product Area	E-mail address
♦ Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products) ♦ Expedite™ 8900 Nucleic Acid Synthesis Systems ♦ PNA Custom and Synthesis ♦ Pioneer™ Peptide Synthesizers ♦ Proteomics Solution 1™ (PS1) Systems ♦ ICAT™ Reagent ♦ FMAT™ 8100 HTS Systems ♦ Mariner™ ESI-TOF Mass Spectrometry Workstations ♦ Voyager™ MALDI-TOF Biospectrometry Workstations ♦ CytoFluor® 4000 Fluorescence Plate Reader	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

To Contact Technical Support by Telephone or Fax (North America)

To contact Applied Biosystems Technical Support in North America, use the telephone or fax numbers in the table below.

Note To schedule a service call for other support needs, or in case of an emergency, dial **1.800.831.6844**, then press **1**.

Product/Product Area	Telephone	Fax
ABI PRISM® 3700 DNA Analyzer	**1.800.831.6844**, then press **8**[a]	**1.650.638.5981**
DNA Synthesis	**1.800.831.6844**, press **2**, then press **1**[a]	**1.650.638.5981**
Fluorescent DNA Sequencing	**1.800.831.6844**, press **2**, then press **2**[a]	**1.650.638.5981**

Product/Product Area	Telephone	Fax
Fluorescent Fragment Analysis (including GeneScan® applications)	**1.800.831.6844**, press **2**, then press **3**[a]	**1.650.638.5981**
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1.800.831.6844**, press **2**, then press **4**[a]	**1.650.638.5981**
ABI PRISM® 3100 Genetic Analyzer	**1.800.831.6844**, press **2**, then press **6**[a]	**1.650.638.5981**
Peptide Synthesis (433 and 43x Systems)	**1.800.831.6844**, press **3**, then press **1**[a]	**1.650.638.5981**
Protein Sequencing (Procise® Protein Sequencing Systems)	**1.800.831.6844**, press **3**, then press **2**[a]	**1.650.638.5981**
Sequence Detection Systems (Real-Time PCR) and PCR	**1.800.762.4001**, then press: **1** for PCR[a] **2** for TaqMan® applications and Sequence Detection Systems including ABI Prism, 7700, 7900, and 5700[a] **6** for the 6700 Automated Sample Prep System[a] or **1.800.831.6844**, then press **5**[a]	**1.240.453.4613**
♦ Mariner™ ESI-TOF Mass Spectrometry Workstations ♦ Voyager™ MALDI-TOF Biospectrometry Workstations ♦ Proteomics Solution 1™ (PS1) Systems ♦ ICAT™ Reagent	**1.800.899.5858**, press **1**, then press **3**[b]	**1.508.383.7855**

Product/Product Area	Telephone	Fax
Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products)	**1.800.899.5858**, press **1**, then press **4**[b]	**1.508.383.7855**
Expedite™ 8900 Nucleic Acid Synthesis Systems	**1.800.899.5858**, press **1**, then press **5**[b]	**1.508.383.7855**
Pioneer™ Peptide Synthesizers	**1.800.899.5858**, press **1**, then press **5**[b]	**1.508.383.7855**
PNA Custom and Synthesis	**1.800.899.5858**, press **1**, then press **5**[b]	**1.508.383.7855**
♦ FMAT™ 8100 HTS Systems ♦ CytoFluor® 4000 Fluorescence Plate Reader	**1.800.899.5858**, press **1**, then press **6**[b]	**1.508.383.7855**
Chemiluminescence (Tropix)	**1.800.542.2369** (U.S. only), or **1.781.271.0045**[c]	**1.781.275.8581**
LC/MS (Applied Biosystems/MDS Sciex)	**1.800.952.4716**	**1.508.383.7899**

a. 5:30 AM to 5:00 PM Pacific time.

b. 8:00 AM to 6:00 PM Eastern time.

c. 9:00 AM to 5:00 PM Eastern time.

To Contact Technical Support by Telephone or Fax (Outside North America)

To contact Applied Biosystems Technical Support or Field Service outside North America, use the telephone or fax numbers below.

Region	Telephone	Fax
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608** or **86 800 8100497**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
India (New Delhi)	**91 11 653 3743/3744**	**91 11 653 3138**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 79588268**	**60 3 79549043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 2358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 532 4484**	**32 (0)2 582 1886**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0)6150 101 0**	**49 (0)6150 101 101**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
Portugal (Lisboa)	**351.(0)22.605.33.14**	**351.(0)22.605.33.15**
Spain (Tres Cantos)	**34.(0)91.806.1210**	**34.(0)91.806.12.06**
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 392400**	**31 (0)180 392409** or **31 (0)180 392499**
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**

Region	Telephone	Fax
European Managed Territories (EMT)		
Africa, English speaking (Johannesburg, South Africa)	**27 11 478 0411**	**27 11 478 0349**
Africa, French speaking (Paris, France)	**33 1 69 59 85 11**	**33 1 69 59 85 00**
India (New Delhi)	**91 11 653 3743** **91 11 653 3744**	**91 11 653 3138**
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 22 866 40 10**	**48 22 866 40 20**
For all other EMT countries not listed (Central and southeast Europe, CIS, Middle East, and West Asia)	**44 1925 282481**	**44 1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6230**	**81 3 5566 6507**
Latin America		
Caribbean countries, Mexico, and Central America	**52 55 35 3610**	**52 55 66 2308**
Brazil	**0 800 704 9004** or **55 11 5070 9654**	**55 11 5070 9694/95**
Argentina	**800 666 0096**	**55 11 5070 9694/95**
Chile	**1230 020 9102**	**55 11 5070 9694/95**
Uruguay	**0004 055 654**	**55 11 5070 9694/95**

To Reach Technical Support Through the Applied Biosystems Web Site

At the Applied Biosystems web site, you can search through frequently asked questions (FAQs) or a solution database, or you can submit a question directly to Technical Support.

Search FAQs

To search for FAQs:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	Click you geographic region for the product area of interest.
4	Follow the instructions under the **Frequently Asked Questions** section (1) to display a list of FAQs for your area of interest.

Search the Solution Database

To search for solutions to problems using the Solution Database:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	Follow the instructions under the **Search the Solution Database** section (2) to find a solution to your problem.

Submit a Question

To submit a question directly to Technical Support:

1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	In the **Personal Assistance – E-Mail Support** section (3), click **Ask Us RIGHT NOW**.
4	In the displayed form, enter the requested information and your question, then click **Ask Us RIGHT NOW**. Within 24 to 48 hours, you will receive an e-mail reply to your question from an Applied Biosystems technical expert.

To Obtain Technical Documents

You can obtain technical documents, such as Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents for free, 24 hours a day. You can obtain documents:

- By telephone
- Through the Applied Biosystems web site

Ordering Documents by Telephone

To order documents by telephone:

1	From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2	Follow the voice instructions to order documents (for delivery by fax). **Note** There is a limit of five documents per fax request.

Obtaining Documents Through the Web Site

To view, download, or order documents through the Applied Biosystems web site:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Documents on Demand**.
3	In the search form, enter and select search criteria, then click **Search** at the bottom of the page.
4	In the results screen, do any of the following: ♦ Click the pdf icon to view a PDF version of the document. ♦ Right-click the pdf icon, then select **Save Target As** to download a copy of the PDF file. ♦ Select the **Fax** check box, then click **Deliver Selected Documents Now** to have the document faxed to you. ♦ Select the **Email** check box, then click **Deliver Selected Documents Now** to have the document (PDF format) e-mailed to you. **Note** There is a limit of five documents per fax request, but no limit on the number of documents per e-mail request.

To Obtain Customer Training Information

To obtain Applied Biosystems training information:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Training**.

References

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

Kwok, S., and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* 339:237–238.

Applied Biosystems
850 Lincoln Centre Drive
Foster City, California 94404-1128
USA

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