Thermo SCIENTIFIC

Lysis Buffer 1 + RNase for Food ID

USER GUIDE

High-throughput purification of PCR-ready DNA from food and feed samples

for use with:
PrepSEQ™ Nucleic Acid Extraction Kit
MagMAX™ Express-96 Deep Well Magnetic Particle Processor
BeadRetriever™ System

Catalog Number A24401
Publication Number MAN0013478
Revision A.0

imegenagro

For testing of Food and Environmental samples only.

ThermoFisher SCIENTIFIC

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

LIMITED USE LABEL LICENSE No. 492: Environmental Testing, Quality Control/Quality Assurance Testing, Food and Agricultural Testing

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) for environmental testing, quality control/quality assurance testing, food and agricultural testing, including reporting results of purchaser's activities in environmental testing, quality control/quality assurance testing, food and agricultural testing for a fee or other commercial consideration. No other right is hereby granted expressly, by implication, or by estoppel. This product is for environmental testing, quality control/ quality assurance testing, food and agricultural testing and research purposes only.

The purchase of this product does not grant the purchaser any additional rights, including (without limitation) the right to transfer or resell the product in any form or the right to use the product as a therapeutic agent or diagnostics test component. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

TRADEMARKS

All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

Contents

About this guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Revision history

Revision	Date	Description
A.0	October 2015	New document. Reformatted Rev. July 2014 from Imegen.

Product information

Product description

The Thermo Scientific™ Lysis Buffer 1 + RNase for Food ID, used with the PrepSEQ™ Nucleic Acid Extraction Kit (Cat. nos. 4480466 or 4428176), enables isolation of total genomic DNA from raw food, processed food, beverages, and feed.

The kit uses magnetic bead technology for reproducible recovery of high-quality DNA in a high-throughput automated workflow. First, samples are homogenized, then treated with RNase and Proteinase K. Samples are placed in one of the recommended automated systems, the MagMAX™ Express-96 Deep Well Magnetic Particle Processor or the BeadRetriever™ System. The Magnetic Particles bind the DNA while RNA and protein are removed in two wash steps. Finally, DNA is eluted and collected, either by magnetic stand or centrifugation, depending on the automated system.

The purified DNA is ready for PCR detection or quantification of specific meat or genetically modified organism (GMO) targets.

Expected DNA yield depends on sample type. See Table 3.

For low-throughput, manual processing, use the GMO Extraction Kit (Cat. no. 4466336).

Kit contents and storage

Table 1 Lysis Buffer 1 + RNase for Food ID (Cat. no. A24401)

Component	Amount (50–100 preps)	Storage[1]
Lysis Buffer 1	4 × 500 mL	15–30°C
RNase	2 × 10 mg	

[1] Refer to the expiration date on the box.

Required materials

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instruments	
One of the following: • MagMAX™ Express-96 Deep Well Magnetic Particle Processor • BeadRetriever™ System	Contact your local sales office.
Equipment	
Hybridization oven or orbital incubator, 65°C	MLS
Benchtop centrifuge, with adapters for 1.5– and 50–mL tubes	MLS
Block heater for 1.5-mL tubes or water bath; 56°C and 100°C	MLS
Homogenizer Laboratory Blender	Cat. no. DB5000A
Magnetic-Ring Stand (96 well)	Cat. no. AM10050
Laboratory scale	MLS
Laboratory mixer (Vortex or equivalent)	MLS
Pipettors	MLS
Plastics and other consumables	
Microcentrifuge tubes (1.5 mL), nuclease-free	MLS
Tubes, nuclease-free (50 mL)	MLS
Micropipette tips, aerosol-resistant	MLS
MagMAX™ Express-96 Deep Well Plates	Cat. no. 4388476
Optional: MagMAX™ Express-96 Standard Plates	Cat. no. 4388475
MagMAX™ Express-96 Deep Well Tip Combs	Cat. no. 4388487
BeadRetriever™ Tubes & Tips	Cat. no. 159-51
Disposable gloves, powder-free	MLS
Plastic paraffin film	MLS
Reagents	
PrepSEQ™ Nucleic Acid Extraction Kit (see Table 2)	Cat. nos. 4480466 and 4428176
Isopropanol	MLS

Item	Source
Ethanol, 95%	MLS
Nuclease-Free Water (not DEPC-treated)	Cat. no. AM9932

Table 2 PrepSEQ™ Nucleic Acid Extraction Kit

Components	Cat. no. 4480466 (100 reactions)	Cat. no. 4428176 (300 reactions)	Storage[1]
Lysis Buffer	2 × 50 mL	6 × 50 mL	15°C to 30°C
Magnetic Particles	2 × 1.5 mL	6 × 1.5 mL	
Binding Solution (Isopropanol)[2]	1 empty bottle	3 empty bottles	
Wash Buffer Concentrate[3]	2 × 26 mL	6 × 26 mL	
Elution Buffer	25 mL	3 × 25 mL	
Proteinase K (PK) Buffer	50 mL	3 × 50 mL	
Proteinase K, 20 mg/mL	1.25 mL	3 × 1.25 mL	–25°C to –15°C

[1] Refer to the product label for the expiration date.
[2] Add ~35 mL of 100% isopropanol to the empty bottle before use.
[3] Add 74 mL of 95% ethanol before use.

Methods

Before first use of the kit

Prepare the RNase

1. Add 1 mL of Nuclease-Free Water to a tube of RNase and incubate at 100°C for 15 minutes.
2. Distribute into aliquots, allow to cool, and store at –20°C. Stable for one year.

Prepare Binding Solution and Wash Buffer

Before using a new PrepSEQ™ Nucleic Acid Extraction Kit, prepare the reagents:

- **Binding Solution**—Add approximately 35 mL of 100% isopropanol to an empty Binding Solution bottle. Label the bottle to indicate that isopropanol is added.
- **Wash Buffer**—Add 74 mL of 95% ethanol to the Wash Buffer Concentrate bottle, and mix well. Label the bottle to indicate that ethanol is added.

Before each use of the kit

Thaw the reagents and preheat the equipment

- Preheat block heater or water bath to 56°C.
- Preheat hybrization oven or orbital incubator to 65°C.
- Thaw RNase, if stored at –20°C. See "Prepare the RNase" on page 8.
- Examine the reagents for a white precipitate, which may have formed if they were stored at a low temperature. Dissolve the precipitate by heating to 50–70°C.

  IMPORTANT! Do not heat Magnetic Particles to 70°C. See "Resuspend Magnetic Particles" on page 9 if there is a white precipitate in the Magnetic Particles.

Resuspend Magnetic Particles

IMPORTANT! Mix the particles vigorously before each use, to ensure that all salts are dissolved.

White precipitate occasionally forms in the Magnetic Particles tube. Extraction experiments show that formation of precipitate does not affect performance as long as the precipitate is fully dissolved prior to use.

1. Incubate the tube of Magnetic Particles at 37±1°C for approximately 10 minutes.
2. Vortex for approximately 10 seconds.

   Note: If the white precipitate is not completely dissolved after 10 minutes at 37°C, apply longer incubation times and higher temperatures (up to 50°C).
3. Keep at room temperature (23±5°C) until ready for use.

Lyse the sample

The starting material should be very fine and homogenous. Grind or homogenize the sample, if necessary.

1. Combine sample and Lysis Buffer 1 in a 50-mL tube, then mix.

Product type	Sample	Lysis Buffer 1
Seeds	20 g	30 mL
Flour, grits, baked goods, meat, fish, snacks, manufactured products	10 g	20 mL
Feed and soy grain	10 g	30 mL
Cocoa, soy flour	5 g	40 mL
Oil, fat, butter	10 mL	20 mL
Dairy products, fruit juice, ice cream, alcoholic beverages	10 mL	10 mL

   Note: If the recommended amount of Lysis Buffer 1 is completely absorbed by the sample, add enough to obtain a solution that can be aspirated with a pipette.
2. Mix RNase thoroughly and add the indicated volume to the sample/Lysis Buffer 1 mixture.
   - Feed and soy products: 20 µL
   - All other sample types: 5 µL
3. Cap the tube, seal with plastic paraffin film, then incubate with shaking at 65°C for 30 minutes.

4. Centrifuge at 3500 × *g* for 5 minutes, then transfer 385 µL of the supernatant to a 1.5-mL tube.

   Alternatively, centrifuge a portion of the sample in a 1.5-mL tube: clean scissors with ethanol, cut the end off a 1-mL pipette tip, and transfer 600–700 µL of sample to a 1.5-mL tube. Centrifuge for 5 minutes and transfer 385 µL of supernatant to a new 1.5-mL tube.

5. Add 12.5 µL of Proteinase K (supplied with the PrepSEQ™ Nucleic Acid Extraction Kit), mix thoroughly, then incubate at 56°C for 1 hour.

   Note: Do not add Proteinase K Buffer.

Bind, wash, and elute the DNA

Prepare Lysis Buffer Premix

This procedure uses reagents from the PrepSEQ™ Nucleic Acid Extraction Kit.

Prepare Mix Solution for the appropriate number of samples:

Component[1]	Volume per sample
Lysis Buffer	250 µL
Magnetic Particles[2]	30 µL
Binding Solution (isopropanol)	300 µL

[1] All components in this premix are from the PrepSEQ™ Nucleic Acid Extraction Kit.

[2] Resuspended and thoroughly mixed.

Proceed immediately to "Process samples on the MagMAX™ Express-96 instrument" on page 10 or "Process samples on the BeadRetriever™ System" on page 11.

Process samples on the MagMAX™ Express-96 instrument

1. Set up the MagMAX™ Express-96 processing plates for the appropriate number of samples:

Plate	Position	MagMAX™ Express-96 plate type	Component	Volume per well
Mix	1	Deep Well	Lysis Buffer Premix[1]	580 µL
Wash	2	Deep Well	Wash Buffer	300 µL
Wash	3	Deep Well	Wash Buffer	300 µL
Elution	4	Standard	Elution Buffer	100 µL[2]
Pickup	5	Standard	—	—

[1] Prepared in "Prepare Lysis Buffer Premix" on page 10.

[2] When using MagMAX™ Express-96, 100 µL of Elution Buffer should be used, regardless of sample type.

   Note: Position 3 includes a drying step.

2. Add 400 µL of each sample to the wells in the Mix Plate.

3. Load all the plates into the instrument, verifying that their orientation is {A1 to A1}.

4. Select script **GMO_MME96_val** and press **Start**.

5. When sample preparation is complete (the message "Remove Elution Plate" is displayed on the screen), remove the Elution Plate.

6. *(Optional)* Place the Elution Plate on the 96-Well Magnetic Ring Stand if Magnetic Particles are present in the eluate. Let the plate stand for 2 minutes, then transfer the eluate, containing genomic DNA, to 1.5-mL tubes.

   Note: Do not store DNA if Magnetic Particles are present.

Proceed directly to real-time PCR, or store DNA in one of the following ways:

- At 5±3°C for up to 24 hours.
- Below –18°C for long-term storage.

Process samples on the BeadRetriever™ System

1. Prepare the BeadRetriever™ Tube Rack for the appropriate number of samples:

Well	Position	Component	Volume per well
Mix	A	Lysis Buffer Premix[1]	580 µL
Wash	B	Wash Buffer	300 µL
Wash	C	Wash Buffer	300 µL
Elution	D	Elution Buffer	50 µL or 100 µL (see Table 3)

[1] Prepared in "Prepare Lysis Buffer Premix" on page 10.

Note: Position C includes a drying step.

Table 3 Elution volume and expected yield

Product type	Elution volume	Expected yield
Starch, corn flour	50 µL	0–10 ng/µL
Sauces	50 µL	0–25 ng/µL
Flavors, colorants	50 µL	0–25 ng/µL
Soups, concentrates	50 µL	0–25 ng/µL
Flour, pasta	100 µL	50–100 ng/µL
Grits	100 µL	25–100 ng/µL
Seeds	100 µL	50–100 ng/µL
Sugars	50 µL	0–10 ng/µL
Meat, fish, coating	100 µL	50–100 ng/µL
Salad, rice, frozen food	50 µL	25–100 ng/µL
Baked goods	50 µL	25–100 ng/µL
Preserves	50 µL	5–50 ng/µL
Soy flour	100 µL	50–100 ng/µL
Cocoa derivatives	50 µL	0–50 ng/µL
Soy lecithin	50 µL	0–10 ng/µL
Oil, fat, butter	50 µL	0–10 ng/µL
Alcoholic beverages	50 µL	0–10 ng/µL
Snacks	50 µL	5–100 ng/µL
Breakfast cereal	50 µL	5–100 ng/µL
Feed	100 µL	50–100 ng/µL
Soy beverages	100 µL	25–100 ng/µL
Dairy products, fruit juice, confections	50 µL	0–100 ng/µL

2. Add 400 µL of sample to the Mix Well.
3. Place the BeadRetriever™ Tube Rack in the BeadRetriever™ instrument, select script **GMO_BR_val**, and press **Start**.
4. Remove the Elution Well when sample preparation is complete.
5. Centrifuge at 11,000 × *g* for 3 minutes, then transfer the clear supernatant, containing genomic DNA, to a new 1.5–mL tube.

   Note: Do not store DNA if Magnetic Particles are present. Remove any remaining particles by placing the tube in a DynaMag™–2 Magnet, then centrifuging, and transferring the liquid to a new 1.5–mL tube.

Proceed directly to real-time PCR, or store DNA in one of the following ways:

- At 5±3°C for up to 24 hours.
- Below –18°C for long-term storage.

A

Troubleshooting

Observation	Possible cause	Recommended action
No DNA, a very low yield of DNA, or poor-quality DNA.	Incomplete sample lysis.	The starting material should be very fine and homogenous. Grind or homogenize the sample, if necessary.
		Mix thoroughly after adding Lysis Buffer 1 and Proteinase K.
	Suboptimal Proteinase K activity.	Ensure correct storage conditions for Proteinase K.
		Use fresh Proteinase K.
	Reagents prepared incorrectly.	See "Prepare the RNase" on page 8 and "Thaw the reagents and preheat the equipment" on page 8.
	Insufficient sample was used.	Repeat the procedure with more sample (see "Lyse the sample" on page 9). It may be necessary to use more than the recommended amount of sample.
		Use a smaller volume of Elution Buffer: • 100 µL if using the MagMAX™ Express-96 • See "Process samples on the BeadRetriever™ System" on page 11 if using the BeadRetriever™ System **Note:** Do not use less than 50 µL of Elution Buffer.
	Sample was taken from the fatty section of food containing multiple textures.	Ensure that the sample for DNA extraction is representative of the whole food, feed, or beverage sample. If the sample contains multiple textures (e.g. lasagna): 1. Cut the sample into small pieces. 2. Homogenize completely. 3. Take a portion of the sample from the aqueous phase if the sample cannot be made uniform. Fat can adversely affect DNA extraction.
DNA is suboptimal for PCR (A_{260}/A_{280} <1.6 or >2.0).	DNA is contaminated with inhibitors (A_{260}/A_{280} <1.6).	Decrease the amount of sample, using the same amount of Lysis Buffer 1 (see "Lyse the sample" on page 9).

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- 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. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of 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.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Food Safety support

Website: **thermoscientific.com/foodmicro** or **thermofisher.com/foodsafety**

Imegen website for Certificates of Analysis and other product documentation: **imegen.es/cms_kits_for_analysis_food.php**

Support email: **foodsafety@lifetech.com**

Phone number in North America: 1-800-500-6855

Phone number outside of North America: Visit **thermofisher.com/support**, select the link for phone support, and select the appropriate country from the dropdown menu.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.