Thermo SCIENTIFIC

GMO Extraction Kit

USER GUIDE

Purification of PCR-ready DNA to test for genetically modified organisms in food and feed

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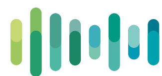

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For testing of Food and Environmental samples only.

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Contents

About this guide 4
Revision history 4
Product information 5
Product description 5
Kit contents and storage 5
Required materials not included with the kit 6
Methods 7
Workflow 7
Before first use of the kit 8
Prepare Proteinase K and RNase 8
Before each use of the kit 8
Thaw the reagents and prepare the materials 8
Lyse the sample 8
Bind and wash the DNA 9
Elute the DNA 10
APPENDIX A Troubleshooting 11
APPENDIX B Safety 13
Chemical safety 14
Biological hazard safety 15
Documentation and support 16
Food Safety support 16
Customer and technical support 16
Limited product warranty 16

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. 15-02-2013 from Imegen.

Product information

Product description

The Thermo Scientific™ GMO Extraction Kit (Cat. no. 4466336) enables isolation of total genomic DNA from raw food, processed food, beverages, and feed.

The kit uses silica-based spin column technology. First, samples are homogenized, treated with RNase and Proteinase K, then applied to a silica-based spin column. The DNA remains bound to the column while RNA and protein are removed in two wash steps. Finally, the purified DNA is eluted from the column.

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 “Elute the DNA“ on page 10.

For high-throughput, automated processing, use Lysis Buffer 1 + RNase for Food ID (Cat. no. A24401) and the PrepSEQ™ Nucleic Acid Extraction Kit (Cat. nos. 4428176, 4480466), in combination with the MagMAX™ Express-96 Deep Well Magnetic Particle Processor or BeadRetriever™ System.

Kit contents and storage

Table 1 GMO Extraction Kit (Cat. no. 4466336)

Component	Amount (50 preps)	Storage[1]
Lysis Buffer 1	2 × 500 mL	15–30°C
Lysis Buffer 2	30 mL	
Wash Buffer 1	30 mL	
Wash Buffer 2	35 mL	
Nuclease-Free Water	30 mL	
Proteinase Buffer	1.8 mL	
Proteinase K	30 mg	
RNase	10 mg	
DNA Filter Columns	50	
Collection Tubes	100	

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

Required materials not included with the kit

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
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, 70°C, and 100°C	MLS
Homogenizer Laboratory Blender	Cat. no. DB5000A
Laboratory scale	MLS
Laboratory mixer (Vortex or equivalent)	MLS
Pipettors	MLS
Plastics and other consumables	
Tubes (1.5 mL and 50 mL), nuclease-free	MLS
Micropipette tips, aerosol-resistant	MLS
Disposable gloves, talcum powder-free	MLS
Plastic paraffin film	MLS
Reagents	
Ethanol, absolute	MLS

Methods

Workflow

Step	Time	Procedure
Lyse the sample	~ 100 minutes	Sample + Lysis Buffer 1 + RNase 65°C ▼ (centrifugation) ▼ Proteinase K, 56°C ▼ Lysis Buffer 2, 70°C ▼ Absolute ethanol ▼
Bind and wash the DNA	~ 5 minutes	(centrifugation) x 2 ▼ Wash Buffer 1 (centrifugation) ▼ Wash Buffer 2 (centrifugation) x 2
Elute the DNA	~ 5 minutes	Nuclease-Free Water ▼ (centrifugation) ▼ Purified DNA

(centrifugation symbol): centrifugation

Before first use of the kit

Prepare Proteinase K and RNase

- **Proteinase K**—Add 1.35 mL of Proteinase Buffer to the vial of Proteinase K and mix well. Store at –20°C; stable for six months.
- **RNase**—Add 1 mL of Nuclease-Free Water to the RNase. Incubate at 100°C for 15 minutes and allow to cool. Store at –20°C; stable for one year.

Before each use of the kit

Thaw the reagents and prepare the materials

- Thaw Proteinase K and RNase if stored at –20°C. See "Prepare Proteinase K and 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.
- Heat block heater or water bath to 56°C.
- Heat hybrization oven or orbital incubator to 65°C.
- Heat Nuclease-Free Water to 70°C. See "Elute the DNA" on page 10 for appropriate volume.
- Assemble filtration columns by inserting DNA Filter Columns into Collection Tubes.

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 the RNase thoroughly and add 20 µL to the sample/Lysis Buffer 1 mixture.
3. Cap the tube, seal with plastic paraffin film, then incubate while 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. *(Optional)* Centrifuge again before transferring the supernatant, if insoluble material is present. See "Columns are saturated. Liquid is not passing through the filter completely." on page 12.
6. Add 25 µL of Proteinase K, mix thoroughly, then incubate at 56°C for 1 hour.
7. Add 400 µL of Lysis Buffer 2, mix thoroughly, then incubate at 70°C for 10 minutes.
8. Add 420 µL of absolute ethanol and mix thoroughly.

Proceed immediately to "Bind and wash the DNA".

Bind and wash the DNA

If necessary, first remove any precipitate that formed after the addition of absolute ethanol. Use a pipette tip.

1. Transfer 600 µL to a DNA Filter Column-Collection Tube assembly and centrifuge at 11,000 × *g* for 1 minute.
2. Discard the liquid in the tube, replace the column in the tube, add the remainder of the sample, then centrifuge at 11,000 × *g* for 1 minute.
3. Discard the tube and insert the column in a new tube, then add 500 µL of Wash Buffer 1 and centrifuge at 11,000 × *g* for 1 minute.
4. Discard the liquid in the tube, replace the column in the tube, add 600 µL of Wash Buffer 2, then centrifuge at 11,000 × *g* for 1 minute.
5. Discard the liquid in the tube, replace the column in the tube, centrifuge again at 11,000 × *g* for 1 minute, then discard the liquid in the tube.

Proceed immediately to "Elute the DNA".

Elute the DNA

1. Insert the DNA Filter Column into a nuclease-free 1.5-mL tube.
2. Add the indicated volume of heated (70°C) Nuclease-Free Water and incubate at room temperature for 3 minutes.

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 derivates	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

3. Centrifuge at 11,000 × *g* for 1 minute to elute DNA.

The purified DNA is in the 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.

Troubleshooting

Observation	Possible cause	Recommended action
No DNA, a very low yield of DNA, or poor-quality DNA (A_{260}/A_{280} <1.6 or >2.0).	Incomplete sample lysis.	Homogenize sample completely.
		Mix thoroughly after adding Lysis Buffer 1 and Proteinase K.
	Suboptimal Proteinase K activity.	Store Proteinase K at –20°C. It is stable for six months.
	Reagents prepared incorrectly.	See "Prepare Proteinase K and RNase" on page 8 and "Thaw the reagents and prepare the materials" on page 8.
	Suboptimal DNA elution.	Ensure that the Nuclease-Free Water used for elution is heated to 70°C.
		Place the Nuclease-Free Water used for elution in the center of the column using a pipette.
		If reagents other than those supplied in the GMO Extraction Kit are used, ensure that the pH is >7.0. A pH <7.0 decreases elution efficiency. Reagents supplied in the GMO Extraction Kit have a pH suitable for elution.
	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 (for example, 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.

Observation	Possible cause	Recommended action
Columns are saturated. Liquid is not passing through the filter completely.	Too much sample was used.	See "Lyse the sample" on page 8 and "Elute the DNA" on page 10.
	Insoluble particles are present.	Check for insoluble material after addition of Lysis Buffer 1 and RNase and centrifugation (see "Lyse the sample" on page 8). If insoluble material is present: 1. Centrifuge again. 2. Transfer 385 µL of supernatant to a new tube. 3. Proceed with addition of Proteinase K.
	A precipitate forms after addition of absolute ethanol.	Remove the precipitate with a pipette tip to allow buffer to pass through the column.
	Incomplete sample lysis.	Homogenize sample completely.
		Mix thoroughly after adding Lysis Buffer 1 and Proteinase K.
	Reagents prepared incorrectly.	See "Prepare Proteinase K and RNase" on page 8 and "Thaw the reagents and prepare the materials" on page 8.
DNA is suboptimal for PCR reactions (A_{260}/A_{280} <1.6 or > 2.0).	Ethanol and salts are not adequately removed.	Follow all centrifugation steps to remove buffers and ethanol.
	DNA is contaminated with inhibitors (A_{260}/A_{280} <1.6).	1. Add 1 volume each of Lysis Buffer 2 and absolute ethanol, and mix thoroughly. 2. Load the mixture into a new filtration column and repeat the procedure from "Bind and wash the DNA" on page 9.

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

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