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GMO Extraction Kit

USER GUIDE

Extraction and purification of DNA from food and feed for next-generation sequencing applications

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Revision B.0

imegenagro

For testing of Food and Environmental samples only.

ThermoFisher SCIENTIFIC

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Revision history: Pub. No. MAN0017943

Revision	Date	Description
B.0	20 May 2020	Removed plant-specific sample buffer.
A.0	08 October 2018	New document.

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Product information

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

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

The purified DNA is ready for quantification, followed by library preparation for next-generation sequencing applications using the appropriate SGS All Species ID kit.

The expected DNA yield depends on sample type.

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 (powder)	30 mg	
RNase[2]	10 mg	

Component	Amount (50 preps)	Storage[1]
DNA Filter Columns	50	15–30°C
Collection Tubes	100	

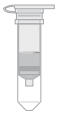
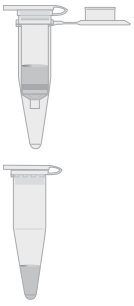
[1] See the expiration date on the box.
[2] Not used in these procedures.

Required materials not included with the kit

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

Item	Source
Equipment	
Benchtop centrifuge, with adapters for 1.5–mL tubes	MLS
Qubit™ 4 Fluorometer	Q33226
Block heater for 1.5-mL tubes or water bath; 56°C, and 70°C	MLS
Homogenizer Laboratory Blender	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

Workflow

Lyse the sample (page 7)	1–16 hours	 	Sample + Lysis Buffer 1 ▼ Proteinase K, 56°C ▼ ⊂⊃ ▼ Lysis Buffer 2, 70°C ▼ Absolute ethanol ▼
Bind and wash the DNA (page 8)	~5 minutes	 	⊂⊃ X2 Wash Buffer 1 ▼ ⊂⊃ ▼ Wash Buffer 2 ▼ ⊂⊃ X2 ▼
Elute the DNA (page 9)	~5 minutes		Nuclease-Free Water, 70°C ▼ ⊂⊃ ▼ Purified DNA

⊂⊃: centrifugation

Methods

Before first use of the kit

Prepare Proteinase K

Add 1.35 mL of Proteinase Buffer to the vial of Proteinase K, then mix well. Store at –20°C; stable for 6 months.

Before each use of the kit

Prepare the materials

- Place Proteinase K on ice until ready to use.
- Examine the reagents for a white precipitate, which can form if the reagents have been stored at a low temperature. Dissolve the precipitate by heating to 50–70°C.
- Heat a block heater or water bath to 56°C.
- Heat a sufficient volume (100 µL per sample) of Nuclease-Free Water to 70°C.
- Assemble filtration columns by inserting DNA Filter Columns into Collection Tubes.

Lyse the sample

Prepare samples as described in the *Thermo Scientific™ NGS Food Authenticity Workflow Guide: Step 1—Sample Homogenization*.

1. Combine 200 mg of prepared sample with the indicated reagents in a 1.5-mL tube.

Sample type	Reagents
Meat, plant, and fish samples	550 µl Lysis Buffer 1

Note: Powdered samples should be gradually added to the lysis buffer to ensure that the sample can move in the liquid. It may be necessary to use less sample (approx. 40mg) or increase the amount of lysis buffer.

2. Add 10 µL of Proteinase K, mix thoroughly, then incubate at 56°C for 1–16 hours.

   All samples should be incubated for 1 hour. If low DNA yields are experienced, incubation of up to 16 hours can be used.

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

4. Transfer the supernatant to a new 1.5-mL microcentrifuge tube.

5. Add 400 µL of Lysis Buffer 2, mix thoroughly, then incubate at 70°C for 10 minutes.

6. Add 420 µL of absolute ethanol, then mix thoroughly.

Proceed immediately to “Bind and wash the DNA” on page 8.

Bind and wash the DNA

If precipitate has formed after the addition of absolute ethanol, remove the precipitate with a pipette tip.

1. Transfer 600 µL (or as much as you can) to a DNA Filter Column-Collection Tube assembly, then centrifuge at 11,000 × *g* for 1 minute.

2. Remove and retain the column from the tube, discard the liquid in the tube, then reinsert the column into the tube. Add the remainder of the sample, then centrifuge at 11,000 × *g* for 1 minute.

3. Remove and retain the column from the tube, discard the used tube, then insert the column into a new tube. Add 500 µL of Wash Buffer 1, then centrifuge at 11,000 × *g* for 1 minute.

4. Remove and retain the column from the tube, discard the liquid in the tube, then reinsert the column into the tube. Add 600 µL of Wash Buffer 2, then centrifuge at 11,000 × *g* for 1 minute.

5. Remove and retain the column from the tube, discard the liquid in the tube, then reinsert the column into the tube. Centrifuge again at 11,000 × *g* for 1 minute.

6. Remove and retain the column in the tube, then proceed immediately to “Elute the DNA” on page 9.

Elute the DNA

1. Insert the DNA Filter Column into a new nuclease-free 1.5-mL tube.
2. Add 100 µL of heated (70°C) Nuclease-Free Water, then incubate at room temperature for 3 minutes.
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 DNA quantification using the Qubit™ fluorometer, or store the 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 (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 6 months.
	Reagents are prepared incorrectly.	See "Prepare Proteinase K" on page 7 and "Prepare the materials" on page 7.
	Suboptimal DNA elution.	Ensure that Nuclease-Free Water used for elution is heated to 70°C.
		Place 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 that are supplied in the GMO Extraction Kit have a pH appropriate 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.
	Longer lysis is required.	Increase incubation time at 56°C in Lysis Buffer 1 and Proteinase K to 16 hours.
	Lysis of plant samples is not optimal.	Use 700 µL CTAB Extraction Buffer + 21 mg of PVPP (Polyvinylpolypyrrolidone) instead of 550 µL of Lysis Buffer 1. CTAB Extraction Buffer: 2% CTAB (Hexadecyltrimethylammonium bromide or Cetyltrimethylammonium bromide), 1.4 M NaCl, 100 nM TRIS, 20 mM EDTA.

Observation	Possible cause	Recommended action
Columns are saturated. Liquid not passed through the filter completely.	Too much sample was used.	See "Lyse the sample" on page 7 and "Elute the DNA" on page 9.
	A precipitate forms after addition of absolute ethanol.	Remove the precipitate with a pipette tip to allow buffer to pass through the column.
	Sample lysis is incomplete.	Homogenize sample completely.
		Mix thoroughly after adding Lysis Buffer 1 and Proteinase K.
	Reagents are prepared incorrectly.	See "Prepare Proteinase K" on page 7 and "Prepare the materials" on page 7.
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 8.

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, and so on). 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. 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also 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:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.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**

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Phone: Visit **thermofisher.com/support**, select the link for phone support, then select the appropriate country from the dropdown list.

Related documentation

Document	Pub. No.
Thermo Scientific™ NGS Food Authenticity Workflow Guide: Step 1—Sample Homogenization	LT2390A Available at the product web page for the SGS All Species ID kits at **thermofisher.com**.

Related products

Table 2 NGS library preparation kits

Kit	Cat. No.	Source
SGS All Species ID Meat DNA Analyser I	A38452	Thermo Fisher Microbiology ordering process
SGS All Species ID Meat DNA Analyser II	A38453	
SGS All Species ID Fish DNA Analyser I	A38454	
SGS All Species ID Fish DNA Analyser II	A38455	
SGS All Species ID Plant DNA Analyser I	A38456	
SGS All Species ID Plant DNA Analyser II	A38457	