

GMO Extraction Kit

USER GUIDE

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

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For testing of Food and Environmental samples only.

ThermoFisher
S C I E N T I F I C



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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

Revision history: MAN0017943 D.0 (English)

Revision	Date	Description
D.0	6 February 2024	<ul style="list-style-type: none">• The manufacturer address was updated.• The trademark statement was updated.• A footnote for Proteinase K was added to the Kit contents and storage table.
C.0	1 February 2022	<ul style="list-style-type: none">• A note was added for Lysis Buffer 1.• Prepare the materials was updated with "Dissolve the precipitate by heating to 56°C for 30 minutes."
B.0	20 May 2020	The plant-specific sample buffer was removed.
A.0	8 October 2018	New document converted from Imegen document for the GMO Extraction Kit.

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

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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 ^[2]
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) ^[2]	30 mg	
RNase ^[3]	10 mg	

Table 1 GMO Extraction Kit (Cat. No. 4466336) (continued)

Component	Amount (50 preps)	Storage ^[1]
DNA Filter Columns	50	15–30°C ^[2]
Collection Tubes	100	

^[1] See the expiration date on the box.

^[2] After reconstitution with the Proteinase Buffer, store the Proteinase K at –20°C.

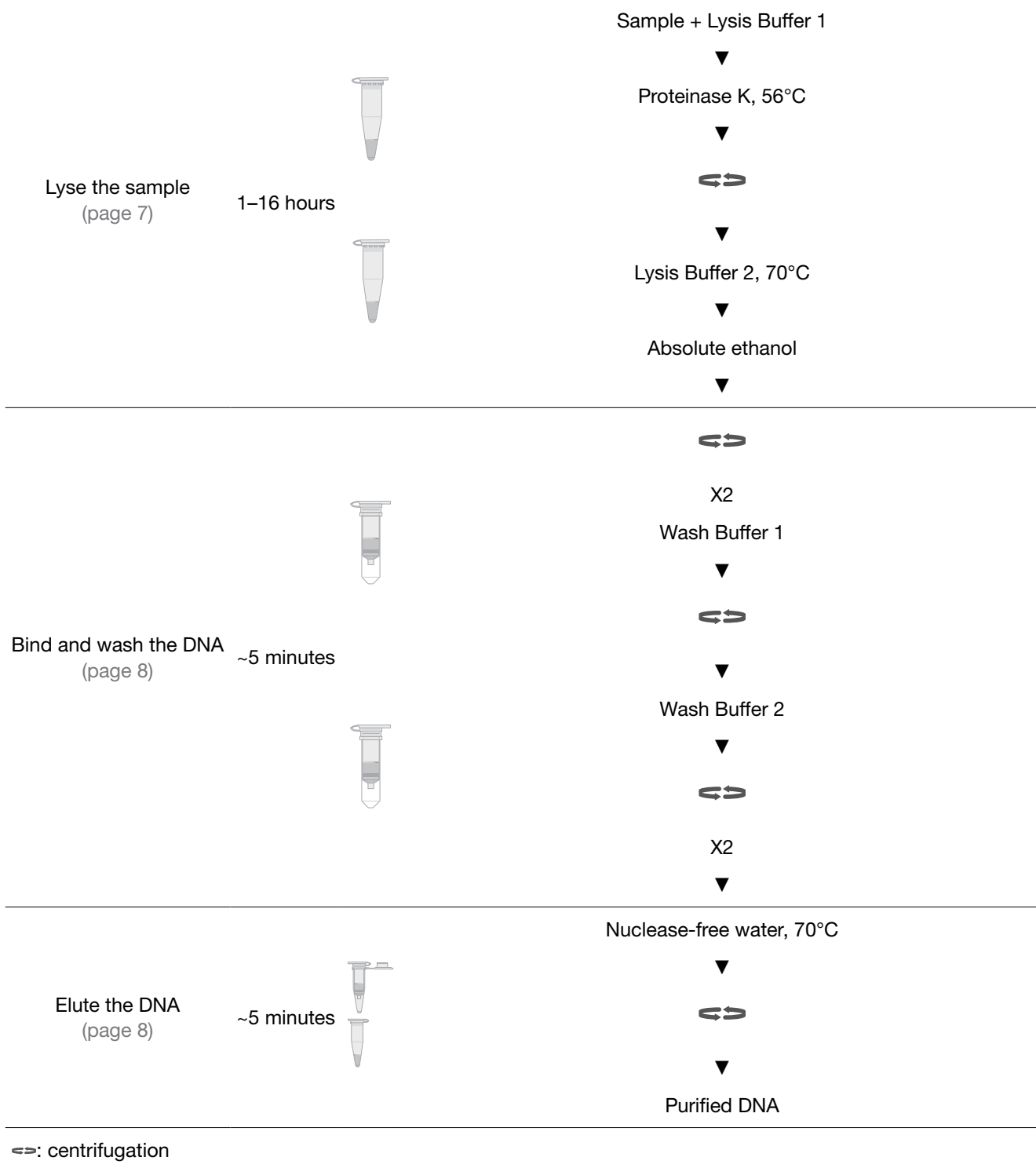
^[3] Not used in these procedures.

Required materials not included with the kit

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or [thermofisher.com](https://www.thermofisher.com). They may also be available through Fisher Scientific ([fisherscientific.com](https://www.fisherscientific.com)), MLS, or another major laboratory supplier.

Item	Source
Equipment	
Benchtop centrifuge, with adapters for 1.5-mL tubes	MLS
Qubit™ 4 Fluorometer	Q33238
Block heater for 1.5-mL tubes or water bath; 56°C, and 70°C	MLS
Homogenizer laboratory blender	MLS
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



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 56°C for 30 minutes.
- 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 (~ 40 mg) 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 $11,000 \times g$ 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 $11,000 \times g$ 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 $11,000 \times g$ 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 $11,000 \times g$ 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 $11,000 \times g$ 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 $11,000 \times g$ for 1 minute.
6. Remove and retain the column in the tube, then proceed immediately to “Elute the DNA” on page 8.

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 $11,000 \times g$ 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 $5 \pm 3^{\circ}\text{C}$ 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 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.

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). (continued)	Lysis of plant samples is not optimal.	Use 700 μ L CTAB Extraction Buffer + 21 mg of PVPP (Polyvinylpolypyrrolidone) instead of 550 μ L of Lysis Buffer 1. CTAB Extraction Buffer: 2% CTAB (Hexadecyltrimethylammonium bromide or Cetyltrimethylammonium bromide), 1.4 M NaCl, 100 nM TRIS, 20 mM EDTA.
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 8.
	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.
DNA is suboptimal for PCR reactions ($A_{260}/A_{280} < 1.6$ or > 2.0).	Reagents are prepared incorrectly.	See “Prepare Proteinase K” on page 7 and “Prepare the materials” on page 7.
	Ethanol and salts are not adequately removed.	Follow all centrifugation steps to remove buffers and ethanol.
	DNA is contaminated with inhibitors ($A_{260}/A_{280} < 1.6$).	<ol style="list-style-type: none"> 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.



Supplemental information

UNE-EN ISO 9001 certification

Health in Code S.L. is certified against the standard UNE-EN ISO 9001:2015 "Quality management systems" for the design, development, manufacture, and commercialization of kits for genetic analysis.

UNE-EN ISO 14001 certification

Health in Code S.L. is certified against the standard UNE-EN ISO 14001:2015 "Environmental Management Systems" for the design, development, manufacture, and commercialization of kits for genetic analysis.



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, visit [thermofisher.com/support](https://www.thermofisher.com/support).



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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311



Documentation and support

Food safety support

Website: <https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html>

Health in Code website for Certificates of Analysis and other product documentation: <https://portal.imegen.es/en/certificate-of-analysis/>

Support email:

- Europe, Middle East, Africa: microbiology.techsupport.uk@thermofisher.com
- North America: microbiology@thermofisher.com

Phone: Visit [thermofisher.com/support](https://www.thermofisher.com/support), select the link for phone support, then select the appropriate country from the dropdown list.

Customer and technical support

Visit [thermofisher.com/support](https://www.thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - 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.



Related documentation

Document	Pub. No.
<i>Thermo Scientific™ NGS Food Authenticity Workflow Guide: Step 1 – Sample Homogenization</i>	LT2390A Available at the product web page for the 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	

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 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.

