February 2020

DNeasy® *mericon*® Food Handbook

For extraction of total nucleic acids from a range of food sample types

Sample to Insight

QIAGEN

Contents

Kit Contents

DNeasy *mericon* Food Kit **Catalog no.** **Number of preps**	**(50)** **69514** **50**
Food Lysis Buffer	4 x 200 ml
Proteinase K	1.4 ml
QIAquick® Spin Columns	50
Buffer PB	2 x 30 ml
Buffer AW2, concentrate	13 ml
Buffer EB	15 ml
Handbook	1

Storage

DNeasy *mericon* Food Kit components should be stored dry at room temperature (15–25°C). The DNeasy *mericon* Food Kit can be stored at 2–8°C, but buffers should be redissolved at 37°C before use, if precipitates are observed. Ensure that all buffers and spin columns are at room temperature (15–25°C) before use.

DNeasy *mericon* Food Kits contain a ready-to-use Proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable for at least 1 year after delivery when stored at room temperature, if not otherwise stated on the label. For storage longer than one year or if ambient temperatures often exceed 25°C, we suggest storing Proteinase K solution at 2–8°C.

DNeasy *mericon* Food Kit components are stable for 1 year under these conditions without showing reduction in performance and quality, if not otherwise stated on the label.

Intended Use

The DNeasy *mericon* Food Kit is intended for molecular biology applications in food, animal feed, and pharmaceutical product testing. This product is not intended for the diagnosis, prevention, or treatment of a disease.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments or to other applicable guidelines.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of DNeasy *mericon* Food Kit is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN® kit and kit component.

CAUTION: Do not add bleach directly to the sample-preparation waste

Buffer PB contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. In case liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.

Introduction

In a globalized food market with increasing demand for food research and monitoring, there is a need for streamlined testing solutions that are sensitive, accurate, and easy to use with a variety of starting materials.

The *mericon* food testing portfolio is a complete system of sample preparation and assay kits that meet the demands listed above. Based on detection by real-time polymerase chain reaction (PCR), *mericon* sample preparation kits and PCR Assays enable fast and reliable detection of a broad range of pathogens, genetically modified organisms, allergens, and plant and animal matter in food, animal feed, or pharmaceutical products.

The DNeasy *mericon* Food Kit is designed for rapid (up to 30 extractions in 2.5 hours) purification of DNA from a variety of raw and processed food matrices, while minimizing the carryover of PCR inhibitors inherent to complex food samples. DNeasy *mericon* purified DNA is ready for use in a real-time PCR using one of the *mericon* PCR Assays.

Principle and procedure

The DNeasy *mericon* Food Kit uses modified cetyltrimethylammonium bromide (CTAB) extraction.

The nonionic detergent CTAB is widely used for efficient extraction of total nucleic acids from a wide range of tissue types. Depending on the salt conditions, CTAB may complex with nucleic acids (low-salt conditions) or complex with inhibitors, such as polysaccharides, proteins, and plant metabolites (high-salt conditions; as found in the Food Lysis Buffer).

The optimized protocols for the DNeasy *mericon* Food Kit use CTAB in combination with Proteinase K to first digest compact tissue and to subsequently precipitate proteins with simultaneous precipitation of other cellular and food-derived inhibitors.

Inhibitors are precipitated by centrifugation, while the extracted DNA remains in solution. In the subsequent chloroform extraction, any remaining CTAB-protein, CTAB-debris, or CTAB-polysaccharide complex not precipitated is removed along with other lipophilic inhibitors into the organic phase. Only the aqueous phase containing the DNA and significantly depleted inhibitors is processed further. This phase is mixed with binding buffer (to adjust binding conditions) and applied to the QIAquick Spin Columns. The DNA obtained is ready for use in a downstream *mericon* real-time PCR assay.

Description of protocols

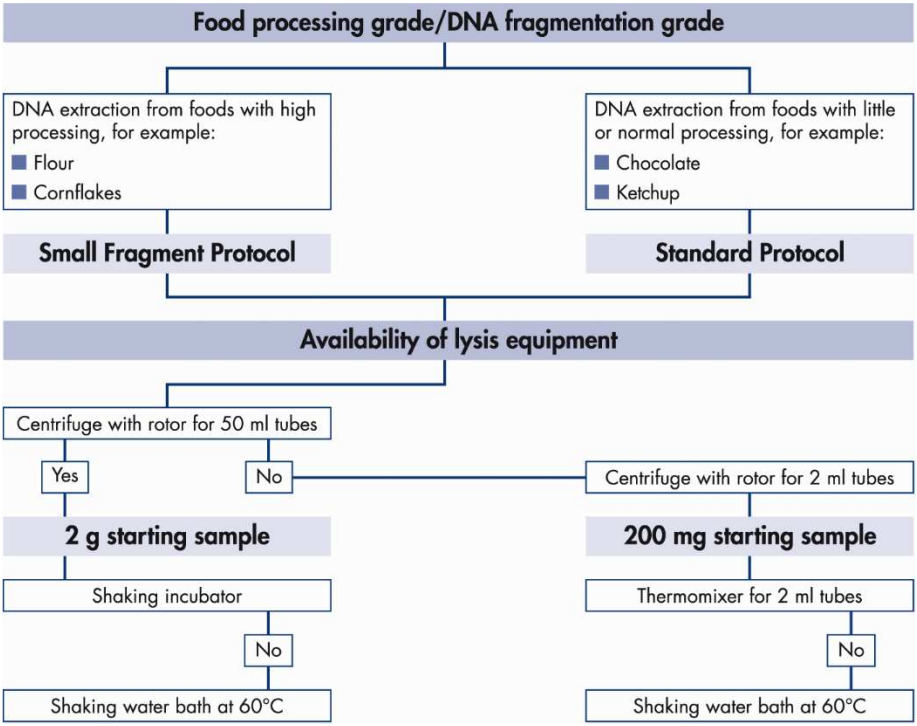
The small fragment protocols are designed for extraction of total DNA from highly processed food material. They have optimized binding conditions for the column purification and are recommended for strongly processed foods, where DNA has been subjected to extensive thermal treatments (e.g., cooking, pasteurization etc.), high pressure, irradiation, pH changes, or drying, and is therefore highly fragmented (down to 100–200 base pairs). These protocols offer more stringent column binding conditions for small DNA fragments and have been successfully used for DNA extraction from foods such as flour and cornflakes.

For all other food types and raw materials, the standard protocols should be used. The standard protocols are designed for the extraction of total DNA from complex food samples for which a lower processing grade and thus less DNA fragmentation is expected. We have successfully extracted DNA from foods such as ketchup and chocolate.

This handbook contains 2 standard and 2 small fragment protocols, depending on the sample input size (large-scale [2 g] or small-scale [200 mg]). Each protocol has scale-specific adjustments. Therefore, sample and input size should be considered for technical prerequisites (see “Equipment and Reagents to Be Supplied by User”, page 10).

However, food samples can be very complex and the processing grade of a specific food is not always clear. It is therefore not possible to predict which protocol will work best for each

sample. The flowchart on the following page may be used as a guide to which protocol is best suited for your sample.



Automated purification of DNA on QIAcube Instruments

Purification of DNA can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the DNeasy *mericon* Food Kit for purification of high-quality DNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

QIAcube Connect.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Standard protocol and small fragment protocol (2 g)

- Homogenizer (see "Important Notes", page 12)
- Vortexer
- Ethanol (96–100%)*
- Chloroform
- Centrifuge tubes (50 ml)
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Centrifuge with rotor for 50 ml tubes
- Microcentrifuge with rotor for 1.5 ml or 2 ml tubes, capable of attaining 17,900 x *g*
- Shaking incubator or shaking water bath capable of attaining 60°C.
- Pipets and pipet tips

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Standard protocol and small fragment protocol (200 mg)

- Homogenizer (see “Important Notes”, page 12)
- Vortexer
- Ethanol (96–100%)*
- Chloroform
- Microcentrifuge tubes (2 ml)
- Microcentrifuge with rotor for 2 ml tubes, capable of attaining 17,900 x *g*
- Thermomixer for 2 ml tubes or shaking water bath capable of attaining 60°C.
- Pipets and pipet tips

Important Notes

Homogenization

Proper disruption of sample material in the protocols is not only important to facilitate food lysis and liberation of DNA, but is also crucial to guarantee a homogeneous starting material, representative of the whole food product.

In this context, anticipated sensitivity and the amount of sample must be considered. The higher the sensitivity requirements (detection of trace amounts of food DNA, e.g., allergens or genetically modified organisms [GMOs]) or the more heterogeneous a food product is (e.g., roughly chopped meats in a sausage), the greater the amount of sample material required for homogenization in order to allow transfer of an overall representative sample into the procedure. In addition to sample size, sample type is also a deciding factor for the homogenization procedure. Both aspects determine the homogenization device best suited for efficient disruption.

In order to select the best homogenization device, it should be determined whether the food is soft, hard, or extremely hard. Several options are available for each type of food (see below).

Soft samples (e.g., whole fruits in fruit jams or vegetables)

- Small amount of starting sample: Small knife mill, QIAGEN TissueRuptor® II, hand blender
- Large amount of starting sample: Large knife mill

Solid/hard samples (e.g., salami or frozen foods)

- Small amount of starting sample: Small knife mill, QIAGEN TissueLyser LT, QIAGEN TissueLyser II, mortar and pestle
- Large amount of starting sample: Large knife mill

Extremely solid/hard samples (e.g., roots or seeds)

- Small amount of starting sample: Small impact mill, QIAGEN TissueLyser LT, QIAGEN TissueLyser II
- Large amount of starting sample: Large impact mill

Disruption using the QIAGEN TissueRuptor II/TissueLyser LT systems

Homogenization using the QIAGEN TissueRuptor II or TissueLyser systems is best carried out in combination with freezing the sample in liquid nitrogen. This ensures optimal homogenization even with difficult sample material.

Disruption using the TissueRuptor II should be carried out without Food Lysis Buffer after freezing the sample in liquid nitrogen. Alternatively, fresh material, such as fruits or vegetables, can be directly disrupted in Food Lysis Buffer without using liquid nitrogen; however, this may cause shearing of high-molecular-weight DNA. We recommend keeping the disruption time to a minimum, to avoid shearing of genomic DNA. With the TissueLyser LT, fresh material can be directly disrupted in lysis buffer without the use of liquid nitrogen. Alternatively, fresh or frozen samples can also be disrupted without lysis buffer after freezing in liquid nitrogen.

We do not recommend disruption of frozen material in lysis buffer as this can result in low yields and degraded DNA.

Protocol: Standard Protocol (2 g)

This protocol is designed for the extraction of total DNA from a large-scale (2 g) sample of raw or processed food material.

Important points before starting

- All centrifugation steps should be carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.

Things to do before starting

- Homogenize the food sample. For information on disruption procedures and suitable disruption devices, see "Important Notes", page 12.
- Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Procedure

1. Place 2 g homogenized food sample in a 50 ml centrifuge tube; add 10 ml Food Lysis Buffer and 25 µl Proteinase K solution. Vortex briefly to ensure complete distribution and moistening of the sample material.

   Note: For samples that swell greatly (e.g., starches), double the amount of Food Lysis Buffer (20 ml) to ensure that sufficient buffer solution covers the sample material.
2. Incubate for 30 min at 60°C with constant shaking. To enhance inhibitor precipitation, cool the sample to room temperature (15–25°C) on ice after incubation.

3. Centrifuge for 5 min at 2500 x *g*.

   Note: The volume of supernatant strongly depends on the nature of the applied starting material and the amount of precipitated CTAB-inhibitor complexes. A range of 2 ml (swelling foods, e.g., homogenized cornflakes) to 7 ml (non-swelling homogenized foods e.g., ketchup) can be expected after centrifugation. Do not carry over any precipitate from the bottom of the tube into the subsequent protocol steps.

4. Pipet 500 µl chloroform into a 2 ml microcentrifuge tube.

   Note: Chloroform is a hazardous substance. Always pipet chloroform in a fume hood.

5. **Note**: As an organic solvent, chloroform may leak from the pipet tip when transferred from one tube to another. This can be avoided by calibrating the pipet tip to the solvent by repeatedly pipetting up and down before transferring a specific volume.

6. Carefully transfer 700 µl of the clear supernatant from step 3 to the microcentrifuge tube containing the chloroform. Be sure not to carry over material from the bottom phase, which contains precipitated food debris.

   Note: The supernatant can be strongly colored. Certain foods may also form three phases after centrifugation. If this happens, go through the upper phase with the pipet and transfer only 700 µl of the clear middle phase. If the upper phase has formed a semi-solid film (for example, as observed with chocolate), pierce the film with the pipet and transfer only 700 µl of the clear middle phase.

7. Vortex the microcentrifuge tube from step 5 vigorously for 15 s and centrifuge at 14,000 x *g* for 15 min.

   Note: If the supernatant is not clear, centrifuge again for 5 min.

8. Pipet 350 µl Buffer PB into a fresh 2 ml microcentrifuge tube, add 350 µl of the upper, aqueous phase from step 6 and mix thoroughly by vortexing.

9. Pipet the solution from step 7 into the QIAquick spin column placed in a 2 ml collection tube. Centrifuge at 17,900 x *g* for 1 min and discard the flow-through. Reuse the collection tube in step 9.

10. Add 500 µl Buffer AW2 to the QIAquick spin column, centrifuge at 17,900 x *g* for 1 min and discard the flow-through. Reuse the collection tube and centrifuge again at 17,900 x *g* for 1 min to dry the membrane.

    Note: Ensure that ethanol is added to Buffer AW2. See "Things to do before starting", page 14.

    Note: Residual ethanol from Buffer AW2 will not be completely removed unless the flow-through is discarded before the additional centrifugation.

11. Transfer the QIAquick spin column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied), and pipet 150 µl Buffer EB directly onto the QIAquick membrane. Incubate for 1 min at room temperature (15–25°C), and then centrifuge at 17,900 x *g* for 1 min to elute.

Protocol: Standard Protocol (200 mg)

This protocol is designed for the extraction of total DNA from a small-scale (200 mg) sample of raw or processed food material.

Important points before starting

- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.
- This protocol uses a smaller starting food sample size. However, overall DNA yields should be similar to those obtained using the standard protocol. This is because supernatants are pooled in step 5 to ensure that the same amount of sample is processed during DNA purification. Be sure to prepare sufficient lysis tubes to be able to pool samples (3–4 tubes).

Things to do before starting

- Homogenize the food sample. For information on disruption procedures and suitable disruption devices, see “Important Notes”, page 12.
- Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Procedure

1. Place 200 mg homogenized food sample in a 2 ml microcentrifuge tube; add 1 ml Food Lysis Buffer and 2.5 µl Proteinase K solution. Vortex briefly to ensure complete distribution and moistening of the sample material.

   Note: To ensure that DNA yields are similar to those obtained using the standard protocol (2 g), supernatants are pooled in step 5. Depending on the starting material, the supernatant from the 1 ml lysis solution will be less than 700 µl. Be sure to prepare sufficient lysis tubes (in the range of 3–4 lysis tubes), so that supernatant aliquots from several lysis tubes can be pooled to draw the 700 µl optimal for subsequent chloroform extraction.

   Note: For samples that swell greatly (e.g., starches), double the amount of Food Lysis Buffer (2 ml) to ensure that sufficient buffer solution covers the sample material.

2. Incubate in a thermomixer for 30 min at 60°C with constant shaking (1000 rpm). To enhance inhibitor precipitation, cool the sample to room temperature (15–25°C) on ice after incubation.

3. Centrifuge for 5 min at 2500 x *g*.

   Note: The volume of supernatant strongly depends on the nature of the applied starting material and the amount of precipitated CTAB-inhibitor complexes. A range of 200 µl (swelling foods, e.g., homogenized cornflakes) to 700 µl (non-swelling homogenized foods e.g., ketchup) can be expected after centrifugation. Make sure not to carry over any precipitate from the bottom of the tube into the subsequent protocol steps.

4. Pipet 500 µl chloroform into a 2 ml microcentrifuge tube.

   Note: Chloroform is a hazardous substance. Always pipet chloroform in a fume hood.

   Note: As an organic solvent, chloroform may leak from the pipet tip when transferred from one tube to another. This can be avoided by calibrating the pipet tip to the solvent by repeatedly pipetting up and down before transferring a specific volume.

5. Carefully draw the maximum volume of clear supernatant from each lysis tube from step 3 without disturbing the inhibitor precipitate at the bottom at the tube. Combine the supernatant aliquots in one microcentrifuge tube and mix by pipetting up and down several times to ensure a homogeneous solution.
6. Transfer 700 µl of the clear supernatant pool to the microcentrifuge tube containing the chloroform.

   Note: The supernatant can be strongly colored. Certain foods may form three phases after centrifugation. If this happens, go through the upper phase with the pipet and transfer only an aliquot of the clear middle phase. If the upper phase has formed a semi-solid film (for example, as observed with chocolate), pierce the film with the pipet and transfer only an aliquot of the clear middle phase.
7. Vortex the microcentrifuge tube from step 6 vigorously for 15 s and centrifuge at 14,000 x *g* for 15 min.

   Note: If the supernatant is not clear, centrifuge again for 5 min.
8. Pipet 350 µl Buffer PB into a fresh 2 ml microcentrifuge tube, add 350 µl of the upper, aqueous phase from step 7 and mix thoroughly by vortexing.
9. Pipet the solution from step 8 into the QIAquick spin column placed in a 2 ml collection tube. Centrifuge at 17,900 x *g* for 1 min and discard the flow-through. Reuse the collection tube in step 10.
10. Add 500 µl Buffer AW2 to the QIAquick spin column, centrifuge at 17,900 x *g* for 1 min and discard flow-through. Reuse the collection tube and centrifuge again at 17,900 x *g* for 1 min to dry the membrane.

    Note: Ensure that ethanol is added to Buffer AW2. See "Things to do before starting", page 14.

    Note: Residual ethanol from Buffer AW2 will not be completely removed unless the flow-through is discarded before the additional centrifugation.

11. Transfer the QIAquick spin column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied), and pipet 150 µl Buffer EB directly onto the QIAquick membrane. Incubate for 1 min at room temperature (15–25°C), and then centrifuge at 17,900 x *g* for 1 min to elute.

Protocol: Small Fragment Protocol (2 g)

This protocol is designed for the extraction of total DNA from a large-scale (2 g) sample of raw or processed food material. It has optimized column binding conditions adjusted for maximal recovery of short DNA fragments. It is recommended for strongly processed foods, where DNA has been subjected to extensive thermal treatments (e.g., cooking, pasteurization etc.), high pressure, irradiation, pH changes, or drying, and is therefore highly fragmented (down to 100–200 base pairs).

Important points before starting

- All centrifugation steps should be carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.

Things to do before starting

- Homogenize the food sample. For information on disruption procedures and suitable disruption devices, see "Important Notes", page 12.
- Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Procedure

1. Place 2 g of homogenized food sample in a 50 ml centrifuge tube; add 10 ml Food Lysis Buffer and 25 µl Proteinase K solution. Vortex briefly to ensure complete distribution and moistening of the sample material.

   Note: For samples that swell greatly (e.g., starches), use double the amount of Food Lysis Buffer (20 ml) to ensure that sufficient buffer solution covers the sample material.

2. Incubate for 30 min at 60°C with constant shaking. To enhance inhibitor precipitation, cool the sample to room temperature (15–25°C) on ice after incubation.
3. Centrifuge for 5 min at 2500 x *g*.

   Note: The volume of supernatant strongly depends on the nature of the applied starting material and the amount of precipitated CTAB-inhibitor complexes. A range of 2 ml (swelling foods, e.g., homogenized cornflakes) to 7 ml (non-swelling homogenized foods e.g., ketchup) can be expected after centrifugation. Make sure not carry over any precipitate from the bottom of the tube into the subsequent protocol steps.
4. Pipet 500 µl chloroform into a 2 ml microcentrifuge tube.

   Note: Chloroform is a hazardous substance. Always pipet chloroform in a fume hood.

   Note: As an organic solvent, chloroform may leak from the pipet tip when transferred from one tube to another. This can be avoided by calibrating the pipet tip to the solvent by repeatedly pipetting up and down before transferring a specific volume.
5. Carefully transfer 700 µl of the clear supernatant from step 3 to the microcentrifuge tube containing the chloroform. Be sure not to carry over material from the bottom phase, which contains precipitated food debris.

   Note: The supernatant can be strongly colored. Certain foods may also form three phases after centrifugation. If this happens, go through the upper phase with the pipet and transfer 700 µl only of the clear middle phase. If the upper phase has formed a semi-solid film (for example, as observed with chocolate), pierce the film with the pipet and transfer only 700 µl of the clear middle phase.
6. Vortex the microcentrifuge tube from step 5 vigorously for 15 s and centrifuge at 14,000 x *g* for 15 min.

   Note: If the supernatant is not clear, centrifuge again for 5 min.
7. Pipet 1 ml Buffer PB into a fresh 2 ml microcentrifuge tube, add 250 µl of the upper, aqueous phase from step 6 and mix thoroughly by vortexing.
8. Pipet 600 µl of the mixture from step 7 into the QIAquick spin column placed in a 2 ml collection tube. Centrifuge at 17,900 x *g* for 1 min and discard the flow-through. Reuse the collection tube in step 9.

9. Repeat step 8 with remaining sample and discard flow through. Reuse the collection tube in step 10.

10. Add 500 µl Buffer AW2 to the QIAquick spin column, centrifuge at 17,900 x *g* for 1 min and the discard flow-through. Reuse the collection tube and centrifuge again at 17,900 x *g* for 1 min to dry the membrane.

    Note: Ensure that ethanol is added to Buffer AW2. See "Things to do before starting", page 21.

    Note: Residual ethanol from buffer AW2 will not be completely removed unless the flow-through is discarded before the additional centrifugation.

11. Transfer the QIAquick spin column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied), and pipet 100 µl Buffer EB directly onto the QIAquick membrane. Incubate for 1 min at room temperature (15–25°C), and then centrifuge at 17,900 x *g* for 1 min to elute.

Protocol: Small Fragment Protocol (200 mg)

This protocol is designed for the extraction of total DNA from a small scale 200 mg sample of raw or processed food material. Column binding conditions are optimized for a maximal recovery of short DNA fragments. It is recommended for strongly processed foods, where DNA has been subjected to extensive thermal treatments (e.g., cooking, pasteurization etc.), high pressure, irradiation, pH changes, or drying, and is therefore highly fragmented (down to 100–200 base pairs).

Important points before starting

- All centrifugation steps should be carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.
- This protocol uses a smaller starting food sample size. However, overall DNA yields should be similar to those obtained using the standard protocol. This is because supernatants are pooled in step 5 to ensure that the same amount of sample is processed during DNA purification. Be sure to prepare sufficient lysis tubes to be able to pool samples (3–4 tubes).

Things to do before starting

- Homogenize the food sample. For information on disruption procedures and suitable disruption devices, see “Important Notes”, page 12.
- Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Procedure

1. Place 200 mg homogenized food sample in a 2 ml microcentrifuge tube; add 1 ml Food Lysis Buffer and 2.5 µl Proteinase K solution. Vortex briefly to ensure complete distribution and moistening of the sample material.

   Note: To ensure that DNA yields are similar to those obtained using the standard protocol (2 g), supernatants are pooled in step 5. Depending on the starting material, the supernatant from the 1 ml lysis solution will be less than 700 µl. Be sure to prepare sufficient lysis tubes (in the range of 3–4 lysis tubes), so that supernatant aliquots from several lysis tubes can be pooled to draw the 700 µl optimal for subsequent chloroform extraction.

   Note: For samples that swell greatly (e.g., starches), double the amount of Food Lysis Buffer (2 ml) to ensure that a sufficient level of buffer solution covers the sample material.

2. Incubate in a thermomixer for 30 min at 60°C with constant shaking (1000 rpm). To enhance inhibitor precipitation, cool the sample to room temperature (15–25°C) on ice after incubation.

3. Centrifuge for 5 min at 2500 x *g*.

   Note: The volume of supernatant strongly depends on the nature of the applied starting material and the amount of precipitated CTAB-inhibitor complexes. A range of 200 µl (swelling foods, e.g., homogenized cornflakes) to 700 µl (non-swelling homogenized foods e.g., ketchup) can be expected after centrifugation. Make sure not carry over any precipitate from the bottom of the tube into the subsequent protocol steps.

4. Pipet 500 µl chloroform into a 2 ml microcentrifuge tube.

   Note: Chloroform is a hazardous substance. Always pipet chloroform in a fume hood.

   Note: As an organic solvent, chloroform may leak from the pipet tip when transferred from one tube to another. This can be avoided by calibrating the pipet tip to the solvent by repeatedly pipetting up and down before transferring a specific volume.

5. Carefully draw the maximum volume of clear supernatant from each lysis tube replicate from step 3 without disturbing the inhibitor precipitate at the bottom at the tube. Combine the supernatant aliquots in one microcentrifuge tube and mix by pipetting up and down several times to ensure a homogeneous solution.
6. Transfer 700 µl of the clear supernatant pool to the microcentrifuge tube containing the chloroform.

   Note: The supernatant can be strongly colored. Certain foods may also form three phases after centrifugation. If this happens, go through the upper phase with the pipet and transfer only an aliquot of the clear middle phase. If the upper phase has formed a semi-solid film (for example, as observed with chocolate), pierce the film with the pipet and transfer only an aliquot of the clear middle phase.
7. Vortex the microcentrifuge tube from step 6 vigorously for 15 s and centrifuge at 14,000 x *g* for 15 min.

   Note: If the supernatant is not clear, centrifuge again for 5 min.
8. Pipet 1 ml Buffer PB into a fresh 2 ml microcentrifuge tube, add 250 µl of the upper, aqueous phase from step 7 and mix thoroughly by vortexing.
9. Pipet 600 µl of the mixture from step 8 into the QIAquick spin column placed in a 2 ml collection tube. Centrifuge at 17,900 x *g* for 1 min and discard the flow-through. Reuse the collection tube in step 9.
10. Repeat step 9 with remaining sample and discard flow through. Reuse the collection tube in step 11.
11. Add 500 µl Buffer AW2 to the QIAquick spin column, centrifuge at 17,900 x *g* for 1 min and the discard flow through. Reuse the collection tube and centrifuge again at 17,900 x *g* for 1 min to dry the membrane.

    Note: Ensure that ethanol is added to Buffer AW2. See "Things to do before starting", page 24.

    Note: Residual ethanol from buffer AW2 will not be completely removed unless the flow-through is discarded before the additional centrifugation.

12. Transfer the QIAquick spin column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied), and pipet 100 µl Buffer EB directly onto the QIAquick membrane. Incubate for 1 min at room temperature (15–25°C), and then centrifuge at 17,900 x *g* for 1 min to elute.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit **www.qiagen.com**).

	Comments and suggestions
Solid film formed on the lysis solution after incubation at 60°C and subsequent centrifugation	
Liberated food components are deposited or compacted on top of the reaction solution after lysis	Continue with the protocol and pierce any top layer with the pipet. Carefully draw the 700 µl aliquot from the clear middle phase, making sure that the pipet tip is not blocked by food deposits.
No supernatant from which to draw the 700 µl aliquot after incubation at 60°C and subsequent centrifugation	
a) Insufficient food disruption and subsequent swelling of food (e.g., cornflakes)	Make sure that the food is completely homogenized before adding the Food Lysis Buffer.
b) Strong swelling of already homogenized food (e.g., starches)	Apply the same amount of sample, but double the amount of Food Lysis Buffer.

	Comments and suggestions
QIAquick membrane is colored	
Food inhibitors carried over from lysis/inhibitor precipitation and chloroform extraction are deposited on the membrane	After washing with Buffer AW2, perform an additional wash step using 500 µl ethanol (96–100%). Centrifuge for 2 min at 20,000 x *g* to dry the membrane and continue with the protocol. In general inhibitors are one of the following: • Retained by the membrane. Membrane remains colored after washing and elution but DNA elution and quality are unaffected • Removed by additional ethanol wash step Co-eluted into DNA solution. See "DNA does not perform well in downstream experiments", below.
DNA eluate is colored	
Inhibitor carryover	See '"DNA does not perform well in downstream experiments", below.
Low DNA yield	
a) Insufficient disruption	Ensure that the starting material is completely disrupted. See "Disruption using the QIAGEN TissueRuptor II/TissueLyser LT systems", page 12.
b) Insufficient lysis	Reduce the amount of starting material and/or increase the amount of Food Lysis Buffer. Check that the correct amount of Proteinase K has been added to the lysis reaction. If necessary, extend incubation time at 60°C for Proteinase K digest to 90 min and/or increase the amount of Proteinase K to 50 µl.
c) Buffer AW2 prepared incorrectly	Make sure that ethanol has been added to Buffer AW2 before use (see "Things to do before starting", pages 17, 21 or 24, depending on which protocol is being used).
d) Incorrect binding conditions	Make sure that the correct amount of lysate has been pipetted after the chloroform extraction and is mixed 1:1 (standard protocol) or 1:4 (small fragment protocol) with Buffer PB to adjust the binding conditions correctly.
e) DNA is still bound to the membrane	Increase the volume of Buffer EB to 200 µl and incubate on the column for 5 min at room temperature (15–25°C) before centrifugation.

	Comments and suggestions
DNA does not perform well in downstream experiments	
a) Inhibitor carryover	A possible inhibitor carryover is sometimes, although not necessarily, identified by a colored eluate. Dilute the sample at least 1:10 before PCR analysis.
b) Ethanol carryover	Ensure that a dry spin step is performed after washing with Buffer AW2. The flow-through after the wash needs to be discarded before the dry spin to allow complete drying of the membrane.
c) Salt carryover	Ensure that Buffer AW2 has been used at room temperature (15–25°C).
d) Insufficient or excess DNA used in downstream application	Optimize the amount of DNA used in the downstream application, if necessary. Downstream applications can be adversely affected by insufficient or excess DNA.

Ordering Information

Product	Contents	Cat. no.
***mericon* Sample Preparation Kits**		
DNeasy *mericon* Food Kit (50)	50 QIAquick Spin Columns, Proteinase K, buffers	69514
mericon DNA Bacteria Kit (100)	Fast Lysis Buffer	69525
mericon DNA Bacteria Plus Kit (50)	50 Pathogen Lysis Tubes L, Fast Lysis Buffer	69534
***mericon* Assay Kits**		
mericon Salmonella spp Kit (24)*	For 24 reactions: *mericon* Salmonella Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect® Nucleic Acid Dilution Buffer, RNase-free water	290013
mericon L. monocytogenes Kit (24)*	For 24 reactions: *mericon* L. monocytogenes Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290023
mericon Campylobacter spp Kit (24)*	For 24 reactions: *mericon* Campylobacter spp. Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290033

Product	Contents	Cat. no.
mericon Campylobacter triple Kit (24)*	For 24 reactions: *mericon* Campylobacter triple Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290043
mericon VTEC stx1/2 Kit (24)*	For 24 reactions: *mericon* VTEC stx1/2 Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290053
mericon Cronobacter spp Kit (24)*	For 24 reactions: PCR Assay Cronobacter spp, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290063
mericon S. aureus Kit (24)*	For 24 reactions: *mericon* S. aureus Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290073
mericon L. monocytogenes Kit (24)	For 24 reactions: PCR Assay L. monocytogenes, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290023

Product	Contents	Cat. no.
mericon Quant L. pneumophila Kit (96)	For 96 reactions: PCR Assay Quant L. pneumophila, Internal Control, Standard DNA, Quant Control DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290095
mericon Shigella spp Kit (24)*	For 24 reactions: *mericon* Shigella spp Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290103
mericon Y. enterocolitica Kit (24)*	For 24 reactions: mericon Y. enterocolitica Assay, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	290113
Related products		
***mericon* GMO Detection Assays**		
mericon Screen 35S Kit (24)*	For 24 reactions: *mericon* Screen 35S Assay, Internal Control, Positive Control, *mericon* Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	291013
mericon Screen Nos Kit (24)*	For 24 reactions: *mericon* Screen Nos Assay, Internal Control, Positive Control, *mericon* Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	291043

Product	Contents	Cat. no.
mericon RR Soy (24)*	For 24 reactions: *mericon* RR Soy Assay, Internal Control, Positive Control, *mericon* Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	291113
***mericon* Animal and Plant Identification Assays**		
mericon Pig Kit (24)*	For 24 reactions: *mericon* Pig Assay, Internal Control, Positive Control, *mericon* Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water	292013
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect†	Instrument, connectivity package, 1 year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395

* Larger kit sizes available; please inquire.

† All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
February 2020	Updated text, ordering information and intended use for QIAcube Connect.

Notes

Notes

Notes

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the DNeasy *mericon* Food Kit to the following terms:

1. The DNeasy *mericon* Food Kit may be used solely in accordance with the DNeasy *mericon* Food Handbook and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the DNeasy *mericon* Food Handbook and additional protocols available at www.qiagen.com.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this Kit and/or its use(s) do not infringe the rights of third-parties.
3. This Kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the Kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Kit and/or its components.

For updated license terms, see **www.qiagen.com**.