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Food Safety

User Guide

Food Extraction Kit

DNA isolation and purification from food, feed or other matrixes

REF : IMG-262

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All products sold by the imegen are subjected to rigorous quality control. The ***Food Extraction kit*** *has passed all internal validation tests, ensuring the reliability and reproducibility of each assay.*

For any questions about the applications of this product or its protocols, please contact our Technical Department:

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1. Product information

Kit description

Food Extraction kit *allows total genomic DNA extraction, as well as mitochondrial DNA, from raw food, processed food, fatty samples (as lecithins, oils, etc.), beverages and feed.*

Our kit is based on a DNA extraction method that allows you to process a large amount of sample (10-20 g) in order to ensure the representativeness of the results.

After the sample preparation and homogenization, it is treated with RNase and Proteinase K, and 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 and it is ready for PCR detection or quantification of specific meat, genetically modified organism (GMO) targets, etc.

Content and storage of the kit

The kit contains needed reagents to perform 50 DNA extraction:

Reagents	Amount
Lysis Buffer 1	2 x 500 mL
Lysis Buffer 2	30 mL
Wash Buffer 1	30 mL
Wash Buffer 2	35 mL
H_2O	30 mL
Proteinase Buffer	1.8 mL
Proteinase K	30 mg

Reagents	Amount
RNase	12 mg
DNA filter columns	50 columns
Collection tubes	100 tubes

Table 1. *Kit components of the* ***Food Extraction Kit***

Equipment and material required but not supplied

In the following table the equipment requirements for using ***Food Extraction Kit*** *are shown:*

Equipment	
1	Desktop centrifuge with adaptors for 1.5- and 50-mL tubes
2	Tube shaker (vortex)
3	Heating block or bath to incubate 1.5-mL tubes at 56°C and 70°C
4	Grinder or other apparatus for sample grinding/homogenisation
5	Hybridisation oven or orbital incubator to incubate at 65°C
6	Micropipettes (10 µl, 20 µl and 200 µl)

Materials	
1	1.5 ml sterile tubes
2	50-mL plastic tubes
3	Disposable micropipette filter tips
4	Absolute ethanol
5	Powder-free latex gloves

Kit specifications

***Food Extraction kit** has been designed to obtain total genomic DNA, as well as mitochondrial DNA from complex samples such as food or feed.*

The DNA thus obtained can be used in PCR processes or in other molecular biology techniques. The main specifications or characteristics of the kit are as follows:

- *Amount of sample to be analysed:*

Product type	Amount of sample	Lysis Buffer 1
Seeds	20 g	30 mL
Flour, grits, baked goods, meats, fish, snacks, manufactured products, etc.	10 g	20 mL
Feed and soy grain	10 g	30 mL
Cocoa, soy flour	5 g	40 mL
Oils, fats, butters	10 mL	20 mL
Dairy products, fruit juices, ice cream, alcoholic drinks	10 mL	10 mL

***Table 2**. Recommended amount of sample to be analysed by type, and amount of lysis buffer 1 required*

- *Estimated preparation time:*

Handling time	30 minutes
Incubation time	100 minutes
Total extraction time	**2 hours**

- *Expected yield of DNA:*

Product type	Expected yield (ng/µL)	Elution volume (µL)
Starch, corn flour	0-10	50
Sauces	0-25	50
Flavours, colorants	0-25	50
Soups, concentrates	0-25	50
Flour, pasta	50-100	100
Grits	25-100	100
Seeds	50-100	100
Sugars	0-10	50
Meats, fish, coatings	50-100	100
Salads, rice, frozen food	25-100	50
Baked goods	25-100	50
Preserves	5-50	50
Soy flour	50-100	100
Cocoa derivatives	0-50	50
Soy lecithin	0-10	50
Oils, fats, butters	0-10	50
Alcoholic drinks	0-10	50
Snacks	5-100	50
Breakfast cereals	5-100	50
Feed	50-100	100
Soy drinks	25-100	100
Dairy products, fruit juices, confectionery	0-100	50

***Table 3**: Expected yields of DNA by type of sample analysed and recommended elution volumes*

2. DNA extraction

Solution preparation

All reagents included in the kit can be stored at room temperature (20–25°C) and are stable for more than one year.

If stored at low temperatures, some of the reagents may form a white precipitate which can easily be dissolved by warming the vials to 50–70°C prior to use.

The following steps should be performed before using the ***FoodExtraction kit****:*

1. *Add 1.35 mL of proteinase buffer to the vial containing proteinase K and store at –20°C (it is stable for at least 6 months under these conditions).*
2. *Add 1.2 mL of water to the tube containing RNase and incubate at 100°C on the heating block for 15 minutes. Allow to cool before storing at –20°C.*

DNA Extraction Protocol

Before commencing the protocol, ensure that:

1. *The RNase and proteinase K solutions have been prepared.*
2. *The heating block has been switched on and set to 56°C.*
3. *The orbital incubator or hybridisation oven has been switched on and the temperature set to 65°C.*

The recommended protocol for DNA extraction is showed below:

1. *Weight the corresponding amount of each homogenised sample (see Table 2) into a 50-mL tube and add the amount of lysis buffer 1 indicated in the same table.*
2. *Shake the tube containing RNase, and add 20 µL to the tube containing*

sample + lysis buffer 1.

3. *Seal each tube with paraffin film and incubate at 65ºC for 30 minutes whilst shaking. An orbital incubator or hybridisation oven should be used for this step.*
4. *Remove the tubes and centrifuge at 3500 g for 5 minutes.*
5. *Add 385 µL of the supernatant to a sterile 1.5-mL tube, then add 25 µL of proteinase K. Shake the tubes and incubate at 56ºC for 1 hour on a heating block or in a water bath.*
6. *Add 400 µL of lysis buffer 2 to each tube. Shake the tubes and incubate at 70ºC for 10 minutes on a heating block or in a water bath.*
7. *Add 420 µL of absolute ethanol. Shake the tubes.*
8. *Transfer 600 µL from each tube to a filtration column (DNA filter column with its corresponding collecting tube). Centrifuge at 11,000 g for 1 min.*
9. *Discard the liquid collected in the collecting tube and add the rest of the sample. Centrifuge at 11,000 g for 1 min.*
10. *Discard the collecting tube and attach a new one.*
11. *Add 500 µL of wash buffer 1 and centrifuge at 11,000 g for 1 min.*
12. *Discard the liquid collected in the collecting tube and add 600 µL of wash buffer 2. Centrifuge at 11,000 g for 1 min.*
13. *Discard the liquid collected in the collecting tube and centrifuge again at 11,000 g for 1 min.*
14. *Place each filtration column in a sterile 1.5-mL tube previously labelled with the sample ID.*
15. *Depending on the type of sample being analysed (see Table 3 in section 4 of this manual), add 50 or 100 µL of sterile ultrapure water preheated to 70ºC in a heating block or bath.*
16. *Incubate at room temperature for 3 minutes.*
17. *Elute the DNA bound to each column by centrifuging at 11,000 g for 1 minute.*

3. Troubleshooting

Problem	Cause/Suggestion
No DNA detected or very low yield.	**1. Incomplete lysis** The sample has not been sufficiently well homogenised and mixed with lysis buffer 1/proteinase K. The sample has not been shaken vigorously after the addition of lysis buffer 1. The activity of proteinase K is not optimal. The diluted proteinase K should be stored for a maximum of 6 months at –20°C. **2. Reagents prepared incorrectly** Prepare the proteinase K and RNase according to the instructions set out in section 2 of this manual. **3. Non-optimal elution of the DNA from the column** Preheat the elution buffer to 70°C before use. Place the buffer in the centre of the column using a pipette. The efficiency of the elution drops markedly if reagents with a pH of less than 7.0 are used.

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Poor quality DNA	***1. Incomplete lysis*** *The sample has not been sufficiently well homogenised and mixed with lysis buffer 1/proteinase K. The sample has not been shaken vigorously after the addition of lysis buffer 1.* *The activity of proteinase K is not optimal. The diluted proteinase K should be stored for a maximum of 6 months at –20ºC.* ***2. Reagents prepared incorrectly*** *Prepare the proteinase K and RNase according to the instructions set out in section 2 of this manual.*
The DNA obtained is not optimal for performing enzymatic reaction	***1. Poor elimination of ethanol or salts*** *Make sure to centrifuge at 11,000 g for more than 1 minute to eliminate wash buffer 2.* ***2. DNA contaminated with inhibitors*** *If the A_{260}/A_{280} absorbance ratio is less than 1.6, the purification process should be repeated (add 1 volume each of lysis buffer 2 and absolute ethanol to the DNA eluted). Load the mixture onto a new column and continue from step 8 of the protocol.*

Columns saturated	***1. Too much starting sample has been used*** *Do not use more sample than recommended in the protocol (see table 2 of this manual).* ***2. Presence of insoluble particles*** *If the sample/lysis buffer 1/RNase mixture contains insoluble material after the first centrifugation step, you are recommended to centrifuge again and transfer the supernatant to a new tube before adding lysis buffer 2 and proteinase K.* ***2. Presence of a precipitate after adding the ethanol*** *Centrifuge the columns several times and, if necessary, remove the precipitate with a pipette to favour the passage of the buffer through the column.* ***3. Incomplete lysis*** *The sample has not been sufficiently well homogenised and mixed with lysis buffer 1/proteinase K. The sample has not been shaken vigorously after the addition of lysis buffer 1.* *The activity of proteinase K is not optimal. The diluted proteinase K should be stored for a maximum of 6 months at –20°C.* ***4. Reagents prepared incorrectly*** *Prepare the proteinase K and RNase according to the instructions set out in section 2 of this manual.*

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Columns saturated (continued)	*The sample has not been sufficiently well homogenised and mixed with lysis buffer 1/proteinase K. The sample has not been shaken vigorously immediately after the addition of lysis buffer 1.* *The activity of proteinase K is not optimal. The diluted proteinase K should be stored for a maximum of 6 months at –20°C.*

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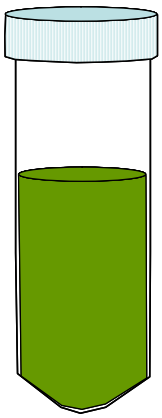


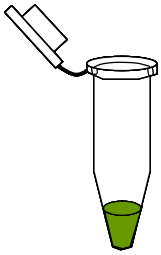
5-20 sample grams

10-20 mL Lysis buffer 1

20 µL RNAse

Incubate 30 minutes at 65°C

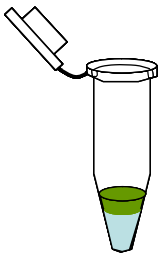
Centrifuge 5 minutes at 3,500 g



385 µL (Sample + Lysis buffer 1)

Add 25 µL Proteinase K

Incubate 60 minutes at 56°C

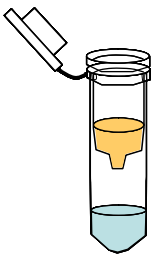


Add 400 µL Lysis buffer 2

Incubate 10 minutes at 70°C

Add 420 µL Ethanol

Centrifuge 1 min at 11,500 g (x2)

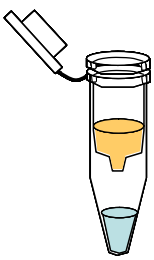


Add 500 µL Wash buffer 1

Centrifuge 1 min at 11,500 g

Add 600 µL Wash buffer 2

Centrifuge 1 min at 11,500 g (x2)



Add 100 µL H_2O at 70°C

Incubate 3 minutes

Centrifuge 1 min at 11,500 g

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