INSTRUCTIONS FOR USE

Imegen® Food Extraction Kit

Ref. IMG-262

Manufactured by HEALTH IN CODE, S.L.
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Guide overview

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

Health in Code, S.L. guarantees that its products are free from defects, both in used materials as in its manufacturing process. This warranty is extended to the expiration date, as long as the storage conditions specified in this manual are met. Our products are designed for research use only. The user of the product is responsible for validating the usefulness of the protocol proposed by Health in Code, S.L. These protocols are considered a guide only. Health in Code, S.L. does not offer any other warranty, express or implied, which extends beyond the proper functioning of the components of this set. Health in Code, S.L. sole obligation in respect of the preceding guarantees, will be to replace the product or return the purchase price thereof, as desired by the customer, as long as the existence of a defect in the materials test, or in the manufacture of its products. Health in Code, S.L. will not be responsible for any damage, direct or indirect, resulting in economic losses or damages resulting from the use of this product by the purchaser or user.

All products sold by Health in Code, S.L. are subjected to rigorous quality control (App. A). The **Imegen® 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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Instructions for Use (IFU) modifications

V.04	JUN 2023	Contents review
V.03	AUG 2022	Change of the manufacturer's identification, going from Imegen to Health in Code, S.L.
V.02	JAN 2020	Contents review

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01

Product information

01.1 General description

The identification of meat, fish and plant species presence in food samples is an essential step in order to verify the origin of the used and traceability of the used raw materials, as well as to evaluate the quality control for the handling and cleaning process of production lines by which it passed.

During the last decade there had been publicly known cases related to the deceptions that consumers suffer at the time of buying meat and receive other species that are not the ones that they are paying for. EU authorities revealed the presence of uncontrolled meat in food products, and because of this, the food industry authorities have developed food safety management systems to improve the resilience of supply chain to food fraud, mostly directed to prevent the fraud opportunity. Despite the fact, food fraud does not impose a health hazard., but in some ways, they are more dangerous because the raw materials and quality control actions are unknown and untraceable.

The possibility to have a fast and accurate method to extract DNA and determine the authenticity of the ingredient used for food preparation is now available but the precision of the results will be something important to take in consideration at the time of evaluating the food processing. The importance of this phenomenon also lies in economic and commercial problems for both the consumer and the production company Customers want to be sure about the origin of the product they are consuming, also the concern of the contained risk for health. DNA analysis allows a valuable and conscious identification of plants and animal derivatives, by efficiently detecting contaminations or fraud related to inaccurate declaration on the label of the species constituting the food.

01.2 Intended use

Imegen® 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.

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, and it is ready for PCR detection or quantification of specific meat, genetically modified organism (GMO) targets, etc.

Imegen® Food Extraction Kit has been designed to obtain whole genomic DNA from complex samples such as foodstuffs and feeds. The DNA obtained can be used to perform PCR reactions or in other molecular biology procedures.

The 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. Expected DNA yield depends on sample type.

01.3 Content and storage conditions of the kit

Imegen® Food Extraction Kit contents the necessary reagents to perform 50 DNA extractions:

Lysis Buffer 1 and 2	Buffers used to break all cell tissues and allow the release of DNA. These buffers contain detergents and chaotropic ions.
Wash Buffer 1 and 2	Wash buffers with alcohols for DNA purification.
Nuclease free water	Used for elution of RNase and extracted DNA.
Proteinase Buffer	Proteinase K stabilizing solution.
Proteinase K	Endopeptidase that destructs proteins in cell lysates and promotes the isolation of nucleic acid.
RNase	Type of nuclease that catalyzes the degradation of RNA into smaller components.
DNA filter columns	Columns that allow the purification of DNA by centrifugation.
Collection tubes	Tubes that collect the solution after passing through the column.

Table 1. IMG-262 Imegen® Food Extraction Kit components and description.

Reagents	Amount	Conservation
Lysis Buffer 1	2 x 500 mL	15-30 °C
Lysis Buffer 2	30 mL	15-30 °C
Wash Buffer 1	30 mL	15-30 °C
Wash Buffer 2	35 mL	15-30 °C
H2O	30 mL	15-30 °C
Proteinase Buffer	1.8 mL	15-30 °C
Proteinase K	30 mg	15-30 °C
RNase	12 mg	15-30 °C
DNA filter columns	50 columns	15-30 °C
Collection tubes	100 tubes	15-30 °C

(*) See the expiration date on the box and tubes.

01.4 Equipment, reagents and materials required but not supplied

Equipment

- Desktop centrifuge with adaptors for 1.5 mL and 50 mL tubes
- Vortex
- Heating block or bath to incubate 1.5 mL tubes at 56˚C, 70˚C and 100˚C
- Grinder or other apparatus for sample grinding/homogenization
- Hybridisation oven or orbital incubator, 65˚C
- Micropipettes (10 µL, 20 µL, 200 µL and 1000 µL)

Materials

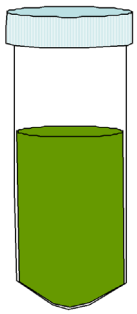
- 1.5 mL sterile tubes
- 50 mL plastic tubes
- Disposable micropipette filter tips (10 µL, 20 µL, 200 µL and 1000 µL)
- Powder-free latex gloves
- Plastic paraffin film

Reagents

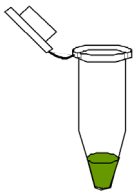
- Absolute ethanol

01.5 Workflow

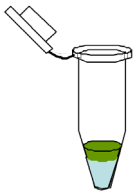
01. Sample Lysis



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

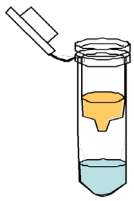


385 µL (Sample + Lysis buffer 1)
Add 25 µL Proteinase K
Incubate 60 minutes at 56°C



Add 400 µL Lysis buffer 2
Incubate 10 minutes at 70°C
Add 420 µL Ethanol

02. DNA wash & bind



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)

03. DNA elution

Add 100 µL H2O at 70°C
Incubate 3 minutes
Centrifuge 1 min at 11,500 g

02 Methods

02.1 Preparation of the reagents

Before first use of the kit:

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

Before each use of the kit:

- Thaw Proteinase K and RNase if stored at -20°C.
- 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 hybridization oven or orbital incubator to 65°C.
- Heat Nuclease-Free Water to 70°C.
- Assemble filtration columns by inserting DNA Filter Columns into Collection Tubes.

02.2 DNA extraction

01. Combine sample and Lysis Buffer 1 in a 50 mL tube (see Table 2), then mix.

Table 2. Recommended amount of sample by type, and amount of lysis buffer 1 required.

Product type	Amount of sample	Lysis Buffer
Seeds	20 g	30 mL
Flour, grits, backed 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

02. Mix the RNase thoroughly and add 20 µL to the sample/Lysis Buffer 1 mixture.

03. Seal each tube with paraffin film and incubate at 65°C for 30 minutes whilst shaking. An orbital incubator or hybridization oven should be used for this step.

04. Remove the tubes and centrifuge at 3500 g for 5 minutes.

05. Add 385 µL of the supernatant to a sterile 1.5 mL tube.

06. Add 25 µL of proteinase K. Shake the tubes and incubate at 56°C for 1 hour on a heating block or in a water bath.

07. Add 400 µL of lysis buffer 2 to each tube. Shake the tubes and incubate at 70°C for 10 minutes on a heating block or in a water bath.

08. Add 420 µL of absolute ethanol, mix thoroughly and proceed immediately to "Bind and wash the DNA".

Bind and wash the DNA:

01. 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.

02. Discard the liquid collected in the collecting tube and add the rest of the sample. Centrifuge at 11,000 g for 1 min.

03. Discard the collecting tube and attach a new one.

04. Add 500 µL of wash buffer 1 and centrifuge at 11,000 g for 1 min.

05. Discard the liquid collected in the collecting tube and add 600 µL of wash buffer 2. Centrifuge at 11,000 g for 1 min.

06. Discard the liquid collected in the collecting tube and centrifuge again at 11,000 g for 1 min.

07. Proceed immediately to "Elute the DNA".

Elute the DNA:

01. Place each filtration column in a sterile 1.5-mL tube previously labelled with the sample ID.
02. Add the indicated volume (see Table 3) of heated (70°C) Nuclease-Free Water.
03. Incubate at room temperature for 3 minutes.
04. Elute the DNA bound to each column by centrifuging at 11,000 g for 1 minute.

Table 3. Expected yields of DNA by type of sample analyzed and recommended elution volumes.

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	20 - 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

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 4ºC for up to 24 hours.
- At -20ºC for long-term storage.

03 Troubleshooting

A list of the potential unexpected results occurred throughout using of Imegen® Food Extraction Kit is included below.

No DNA, a very low yield of DNA, or poor-quality DNA (A_{260}/A_{280} < 1.6 or > 2.0):

Possible cause	Recommended action
Incomplete sample lysis	Homogenize sample completely.
	Mix thoroughly after adding Lysis Buffer 1 and Proteinase K.
Suboptimal Proteinase K activity	Store Proteinase K at -20ºC. It is stable for six months.
Reagents prepared incorrectly	See sections 02.1 of this document.
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 Food Extraction Kit are used, ensure that the pH is > 7.0. A pH < 7.0 decreases elution efficiency.
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): 01. Cut the sample into small pieces. 02. Homogenize completely. 03. Take a portion of the sample from the aqueous phase if the sample cannot be made uniform. Fat can adversely affect DNA extraction.
Ethanol and salts are not adequately removed	Follow all centrifugation steps to remove buffers and ethanol

DNA is contaminated with inhibitors (A260/A280 < 1.6).	01. Add 1 volume each of Lysis Buffer 2 and absolute ethanol and mix thoroughly. 02. Load the mixture into a new filtration column and repeat the procedure from section 02.2 (Bind and wash the DNA) of this document.

Columns are saturated. Liquid is not passing through the filter completely:

Possible cause	Recommended action
Too much sample was used	See section 02.2 of this document.
Insoluble particles are present	Check for insoluble material after addition of Lysis Buffer 1 and RNase and centrifugation (see section 6.3 of this document). If insoluble material is present: 01. Centrifuge again. 02. Transfer 385 µL of supernatant to a new tube. 03. 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 section 02.1 of this document.

04 Limitations

04.1 Product Stability

The optimal analytical functioning of this product is confirmed as long as the recommended storage conditions are applied as specified on Section 01.3 (Contents and Storage Conditions) from the reception of the kit until the expiry date assigned to each production batch.

Appendix A

Supplemental information

AA.1 Quality certifications

- Health in Code, S.L. is certified by IVAC against the standard UNE-EN ISO 9001:2015 "Quality management systems" for the design, development, manufacture, and commercialization of kits for genetic analysis (Certification number ES090493-1).
- 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.

Appendix B

Safety warnings and precautions

	Strictly follow the instructions of this manual, especially regarding the handling and storage conditions.
	Do not pipette by mouth.
	Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
	You must properly protect any skin condition, as well as cuts, abrasions and other skin lesions.
	Send down the drain only those materials found on the safe list. Compounds not listed are not suitable for drain disposal. Use waste containers according to the local legislation and manage their treatment through an authorised waste manager.
	In case of an accidental release of any of the reagents, avoid contact with skin, eyes and mucous membranes and clean with abundant water.
	The materials safety data sheets of all hazardous components contained in this kit are available on request to Health in Code, S.L.
	This product could require the handling of samples and materials of human and animal origin. You should consider all human and animal source materials as potentially infectious and handled in accordance with OSHA Biosafety Level 2 of bloodborne pathogens or must use other relevant biosafety practices for materials containing or suspect that they may contain infectious agents.

Reagents included in this kit are non-toxic, neither explosive, infectious, radioactive, magnetic, corrosive nor environmental polluters.

This kit has been validated with specific equipment under certain conditions, which could be different in other laboratories. It is recommended that each laboratory performs an internal validation when the kit is used for the first time.

The manufacturer is not responsible for the malfunction of the assay when one or more reagents included in the kit are replaced by other reagents not supplied by Health in Code, S.L.

The manufacturer does not guarantee the reproducibility of the assay when the user employs reagents not validated by Health in Code, S.L., considering them equivalent to those provided in the kit.

Appendix C

Documentation and support

AC.1 Food safety support

Please, visit our website for the latest services, orders and support information:

imegenagro.es

Health in Code certificates of analysis and other product documentation:

portal.imegen.es/en/certificate-of-analysis/

AC.2 Customer and technical support

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

+34 963 212 340

tech.support@healthincode.com

NOTE: For SDSs for reagents and chemicals from other manufacturers, please contact the appropriate manufacturer.
