Instructions for Use
Life Science Kits & Assays

PME Food DNA Kit

iST Innuscreen GmbH

Order No.:
845-IR-0008050 50 reactions

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Contents

1 Introduction

1.1 Intended use

The content of DNA in liquid or instant foods is usually very low and the DNA is highly degraded. Because of these facts, the extraction of DNA from such foods is difficult.

The PME Food DNA Kit is based on a new technology, called: PME – Polymer Mediated Enrichment. This Kit is designed to process liquid food sample and solid food sample.

In first step, if necessary, the instant samples have to be dissolved in distilled water. Then proteins have to be digested with Proteinase K. Next step is capturing of cell-free DNA with a special polymer. Subsequently the captured DNA, cells and cell debris are dissolved in a lysis buffer followed by lysis. After lysis step DNA is bound on spin filter, washed and eluted.

Solid food sample can be directly lysed without any pre-treatment and the DNA can be bound on spin filter. Then the bounded DNA is washed and eluted.

Please note that the eluates of the kit contain both sample DNA and Carrier Mix. Therefore, it is not possible to quantify the isolated nucleic acids by photometric or fluorometric methods when using the Carrier Mix. Therefore, other methods for quantification such as specific quantitative PCR or Real-time PCR systems are recommended. Furthermore, Carrier RNA may inhibit PCR reactions. The amount of added Carrier RNA may thus be carefully optimized depending on the individual PCR system used.

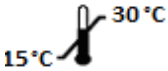
The kit has been designed to be used for a wide range of different downstream applications, like amplification reactions and further analytical procedures.

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions.

1.2 Notes on the use of this manual

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number.
	**Content** Contains sufficient reagents for <N> tests.
	**Storage conditions** Store at room temperature or shown conditions respectively.
	**Consult instructions for use** This information must be observed to avoid improper use of the kit and the kit components.
	**Expiry date**
	**Lot number** The number of the kit charge.
	**Manufactured by** Contact information of manufacturer.
	**For single use only** Do not use components for a second time.
	**Note / Attention** Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual" p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
Don't eat or drink components of the kit!
The kit shall only be handled by educated personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation

Please observe the federal, state and local safety and environmental regulations. Observe the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!
Do not add bleach or acidic components to the waste after sample preparation!

NOTE
Emergency medical information in English and German can be obtained

24 hours a day from:
Poison Information Centre, Freiburg / Germany
Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please dial +49 30 9489 3380.

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3 Storage conditions

Store **Enrichment Reagent VCR-1** at 4 ℃ to 8 ℃.

Store lyophilized **Proteinase K** at 4 ℃ to 8 ℃. Aliquot dissolved **Proteinase K** and store at -22 ℃ to -18 ℃. Repeated freezing and thawing will reduce the activity dramatically!

Store lyophilized and dissolved **Carrier Mix** at -22 ℃ to -18 ℃. Aliquot dissolved **Carrier Mix** and do not freeze and thaw it more than 3 times!

All other components of the PME Food DNA Kit should be stored dry, at room temperature (15 ℃ to 30 ℃). Under this condition the kit is stable until the expiration date printed on the label on the kit box.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming.

4 Function testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This kit was produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the PME Food DNA Kit, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 30 9489 3380.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Product specifications” p. 9). Since the performance characteristics of our kits have not been validated for any specific application. IST Innuscreen GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by the IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
For research use only!

6 Kit components

6.1 Included kit components

	Σ 50
REF	845-IR-0008050
Proteinase K	for 2 x 1.5 ml working solution
Carrier Mix	for 1.25 ml working solution
RNase-free Water	2 ml
Enrichment Reagent VCR-1	2 x 1.2 ml
Enrichment Reagent VCR-2	10 ml
Lysis Solution SLS	60 ml
Binding Solution SBS	12 ml
Washing Solution MS (conc.)	24 ml
Elution Buffer	10 ml
Spin Filter	50
Receiver Tubes	4 x 50
Elution Tubes	1 x 50
Manual	1

6.2 Components not included in the kit

- 2 ml tubes
- 96–99.8 % ethanol (molecular biology grade, undenaturated)
- ddH_2O for dissolving **Proteinase K**

6.3 Related products

Products for Animal DNA Detection	Order No. (for 24 reactions)
innuDETECT Pork Assay	845-IDF-0010024
innuDETECT Beef Assay	845-IDF-0020024
innuDETECT Horse Assay	845-IDF-0030024
innuDETECT Goat Assay	845-IDF-0040024
innuDETECT Sheep Assay	845-IDF-0050024
innuDETECT Chicken Assay	845-IDF-0060024
innuDETECT Turkey Assay	845-IDF-0070024
innuDETECT Donkey Assay	845-IDF-0080024
innuDETECT Mammal & Bird Assay	845-IDF-0090024
innuDETECT Fish Assay	845-IDF-0100024
innuDETECT Cheese Assay	845-IDF-0110024
innuDETECT Halal Assay	845-IDF-0120024
innuDETECT Halal Multiplex Assay	845-IDF-0130024

7 Usage of Carrier Mix

■ Besides carrier RNA, the **Carrier Mix** contains an Internal Control DNA and RNA (IC DNA and IC RNA). Both can be detected by real-time PCR using the corresponding assays.

Name	Amount	Order No.
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100

Add these components to the samples to be extracted as described in the protocol. (→ "Extraction from liquid and water-dissolvable samples" p.11 and "DNA Extraction from solid food samples" p. 14).

8 Product specifications

1. **Starting material:**

- 1 ml liquid sample or appropriate amount of dissolvable sample in water
- Up to 100 mg solid food sample

NOTE
Avoid freezing and thawing of starting material.

2. **Time for isolation:**

- Approximately 2.5 hours for liquid sample including enrichment step
- Approximately 1.5 hours for solid food sample

3. **Typical yield:**

- Depending on sample and amount of starting material

9 Initial steps before starting

- Heat thermal mixer or water bath at 50 °C for Proteinase K digestion step (only necessary when handling liquid samples) and later at 70 °C for lysis of sample.
- Add 1.5 ml ddH_2O to each vial lyophilized **Proteinase K**, mix thoroughly and store as described above.
- Add 1.25 ml RNase-free water to lyophilized **Carrier Mix**, mix thoroughly and store as described above.
- Add 56 ml ethanol to the **Washing Solution MS (conc.)**, mix thoroughly. Store as described above and always keep the bottle firmly closed!
- Centrifugation steps should be carried out at room temperature.

10 DNA extraction protocols

10.1 Extraction from liquid and water-dissolvable samples

1. Pipette the **liquid sample** or **weigh the food sample** and dissolve in ddH_2O into a 2.0 ml tube as described in table below.

Kind of food sample	Amount	Dissolving in ddH_2O
Milk, liquid products (Juice, vinegar, wine, cream, shake etc.)	1.0 ml	no
Thick milk products (yoghurt, condensed milk, kefir)	1.0 g	300 µl
Dry instant products without or with low amount of milk (3 in1 coffee, baby milk powder, dry milk etc.)	0.6 g	1000 µl
Gelatine	0.4 g	1000 µl
Gelatine containing foods	1.0 g	1000 µl

NOTE
Dissolve the sample by inverting the tube or by vortexing if necessary.

2. Add **30 µl Proteinase K and 10 µl Carrier Mix** to each sample and vortex vigorously for 10 seconds. Incubate at 50 °C for 30 min under continuous shaking of the sample.
3. Add **30 µl of Enrichment Reagent VCR-1** and vortex shortly. Add **150 µl of Enrichment Reagent VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 10 minutes.
4. Centrifuge at maximum speed for 10 minutes (at least 11.000 x g), open the tube and remove the supernatant and floating material above the sample carefully as much as possible.

NOTE
Do not remove the pellet.

5. Add **1 ml ddH_2O**, invert the reaction tube three times and centrifuge at maximum speed for 1 minute, open the tube and remove the supernatant carefully as much as possible.

NOTE
Note: Don't remove the pellet; it will be processed like the following steps!

6. Add **400 µl Lysis Solution SLS** to the reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times.
7. Add **25 µl Proteinase K** to the reaction tube and mix vigorously by pulsed vortexing for 10 seconds. Incubate at 70 °C for 60 minutes under continuously shaking at 1,000 rpm.
8. Centrifuge the tube at maximum speed for 2 minutes.
9. Transfer the supernatant without disturbing the pellet into a new 2.0 ml reaction tube.

NOTE
If there is a floating material above the sample, pierce this film carefully with pipette and carefully remove the sample. Avoid aspiration of floating material and/or sediment.

10. Add **200 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

NOTE
It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

11. Apply the whole sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11.000 x g (~12.000 rpm) for 2 minutes.
12. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

13. Open the Spin Filter and add **500 µl Washing Solution MS**, close the cap and centrifuge at 11.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
14. Open the Spin Filter and add **800 µl Washing Solution MS,** close the cap and centrifuge at 11.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
15. Centrifuge at maximum speed (at least 11.000 x g) for 3 minutes to remove all residues of ethanol. Discard the 2.0 ml Receiver Tube.
16. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **100 µl Elution Buffer**. Incubate at room temperature for 1 minute. Centrifuge at 11.000 x g (~12.000 rpm) for 1 minute. Two elution steps with equal volumes of Elution Buffer (e.g. 50 µl + 50 µl) might increase the yield of extracted DNA.

NOTE
Store DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C!

10.2 DNA Extraction from solid food samples

Food class	Example
Meat products	ham, salami
Tinned food	fish, meat or sausages
Milk products	cheese, chocolate
Cereals	flakes, nachos, waffle, cookie, noodle
Flours	wheat flour, Cornmeal, Soybean meal
Instant products	Instant soups, mashed potatoes
Bread	white bread

1. Weigh up to **100 mg** of solid food sample, cut the sample in small pieces or homogenize the sample as much as possible and transfer it into a 2.0 ml tube.
2. Add **1 ml of Lysis Solution SLS, 25 µl Proteinase K** and **10 µl Carrier Mix** to each sample and vortex vigorously for 10 seconds. Incubate at 70 ˚C for 60 minutes under continuously shaking at 1,000 rpm.
3. Centrifuge the tube at maximum speed (at least 11.000 x g) for 2 minutes.
4. Transfer 400 µl of supernatant into a new 2.0 ml reaction tube.

NOTE

If there is a floating material above the sample, pierce this film carefully with pipette and carefully remove the sample. Avoid aspiration of floating material and/or sediment.

5. Add **200 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

NOTE

It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

6. Apply the whole sample to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11.000 x g (~12.000 rpm) for 2 minutes.
7. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
8. Open the Spin Filter and add **500 µl Washing Solution MS**, close the cap and centrifuge at 11.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
9. Open the Spin Filter and add **800 µl Washing Solution MS,** close the cap and centrifuge at 11.000 x g (~12.000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
10. Centrifuge at maximum speed (at least 11.000 x g) for 3 minutes to remove all residues of ethanol. Discard the 2.0 ml Receiver Tube.
11. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **100 µl Elution Buffer**. Incubate at room temperature for 1 minute. Centrifuge at 11.000 x g (~12.000 rpm) for 1 minute. Two elution steps with equal volumes of Elution Buffer (e.g. 50 µl + 50 µl) might increase the yield of extracted DNA.

NOTE
Store DNA under adequate conditions. We recommend storing the extracted DNA at -22 °C to -18 °C!

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Too small amount (< 400 µl) of Lysis Solution SLS after Lysis is left	
The sample is too absorbent	Add. more Lysis Solution SLS (up to 1,5 ml) to the Sample
No pellet after enrichment step	
Insufficient addition of **VCR-1** or **VCR-2**	Make sure that both **VCR-1** and **VCR-2** are added to the reaction tube. Make sure that the right volume of **VCR-1** and **VCR-2** are added.
Insufficient centrifugation	Make sure that centrifugation steps are carried out as describe in the manual. Otherwise repeat centrifugation
Removing of pellet	Ensure that the pellet is not discarded during removing the supernatant. In some cases, the pellet is not been seen until the supernatant is removed completely.
Pellet is difficult to dissolve	
Lysis solution not enough added to pellet	Ensure that lysis solution is pipette as described in protocol.
Pipette tip is clogged while dissolving the pellet	Cut the slide edge of pipette tip and try to transfer the pellet as much as possible.
Clogged Spin Filter	
Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.
Inhibition effects in the subsequent PCR reaction	
Impurities in the sample were not washed out sufficiently	Perform an additional wash step (after the 2x wash with Washing Solution MS) with 700 µl of 80% ethanol

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