Instructions for Use
Life Science Kits & Assays

PME Gelatin DNA Kit

iST Innuscreen GmbH

Order No.:

845-IR-0007010 10 reactions
845-IR-0007050 50 reactions

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1 Introduction

1.1 Intended use

Assurance of optimal food quality and meeting international standards are important challenges in food stuff industry. Not least this includes identification of not declared constituents from animal origin. Poor quality and quantity of DNA extracted from gelatin and gelatin containing foods often causes failure in the determination of animal species using PCR.

The content of DNA in gelatin is usually very low and the DNA is highly degraded. Because of these facts, the extraction of DNA from gelatin and gelatin containing foods is difficult. The PME Gelatin DNA Kit is based on a new technology, called: PME – Polymer Mediated Enrichment.

The procedure starts with dissolving of gelatin containing sample and digestion of protein. The next step is capturing of cell free DNA with a special polymer. Subsequently the captured DNA is dissolved in a lysis buffer followed by removal of impurities by precipitation.

The kit contains a Carrier RNA/DNA Mix. Addition of Carrier Mix is recommended if extreme low amount of DNA is expected. In this case the addition of Carrier Mix can increase the final yield. The artificially DNA inside of Carrier Mix can be used to evaluate nucleic acid extraction and to verify the absence of inhibitors during amplification.

The kit is intended for use by professional users. 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 before use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual and the kit

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

Symbol	Information
REF	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> tests.
15 °C 30 °C	**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	**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 before 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. IST Innuscreen GmbH has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely, but cannot be excluded completely. 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 contact sds.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved **Proteinase K** and **Enrichment Reagent VCR-1** at 4 °C to 8 °C!

Dissolved **Carrier Mix** has to be stored at -22 °C to -18 °C.

All other components of the **PME Gelatin DNA Kit** should be stored dry, at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

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

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been 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 Gelatin DNA Kit** or other IST Innuscreen GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. For other countries please contact your local distributor.

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 (→ “Intended use” p. 2) (→ “Product specifications” p. 9). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. 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 equivalent regulations required 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
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 10	Σ 50
REF	845-IR-0007010	845-IR-0007050
Proteinase K	For 2 x 0.3 ml working solution	For 2 x 1.5 ml working solution
Carrier Mix	For 1.25 ml working solution	For 1.25 ml working solution
RNase-free Water	2 ml	2 ml
Enrichment Reagent VCR-1	1.2 ml	2 x 1.2 ml
Enrichment Reagent VCR-2	2 ml	10 ml
Lysis Solution CBV	5 ml	25 ml
Precipitation Buffer P	2 ml	2 x 2 ml
Washing Solution MS (conc.)	15 ml	60 ml
Elution Buffer	2 ml	3 x 2 ml
Spin Filter	10	50
Receiver Tubes	50	4 x 50
Elution Tubes	2 x 10	2 x 50
Manual	1	1

6.2 Components not included in the kit

- 2 ml tubes
- 96-99.8 % ethanol (molecular biology grade, undenaturated)
- RNase-free Water and ddH_2O for dissolving **Carrier Mix** and **Proteinase K**
- Propan-2-ol (Isopropanol)

8 Product specifications

1. Starting material:
   - Gelatin powder or sheets up to 0.5 g
   - Gelatin containing food up to 1 g

2. Time for isolation:
   - Approximately 2 hours including dissolving and lysis steps

3. Typical yield:
   - Depending on sample and amount of starting material

9 DNA isolation from gelatin or gelatin containing food samples

1. Add up to **0.5 g gelatin** powder or sheet or up to **1 g gelatin** containing food into a 2.0 ml reaction tube.
2. Add **1 ml ddH_2O, 30 µl Proteinase K** and **10 µl of dissolved Carrier Mix**, mix vigorously by pulsed vortexing for 10 seconds and incubate the sample at 50 °C for 30–45 minutes.

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3–4 times during the incubation. No shaking will reduce the lysis efficiency.

3. Centrifuge the 2.0 ml reaction tube at 11,000 x g (~12,000 rpm) for 30 seconds to remove the non-dissolved parts. Transfer the supernatant into a new 2.0 ml tube.
4. Open the 2.0 ml reaction tube and add **30 µl of Enrichment Reagent VCR-1**. Mix shortly by vortexing, add **150 µl of Enrichment Reagent VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 10 minutes.
5. Centrifuge at maximum speed for 10 minutes, open the tube and remove the supernatant carefully as much as possible.
6. Add **2 ml ddH_2O**, invert the reaction tube three times and centrifuge at maximum speed for 5 minutes, open the tube and remove the supernatant carefully as much as possible.
7. Add **400 µl Lysis Solution CBV** to the reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times. Try to avoid thereby the formation of air bubbles!
8. Incubate at 70 °C for 15 minutes under continuously shaking at 1,000 rpm.
9. Add **75 µl Precipitation Buffer P**. Incubate at room temperature for 5 minutes.

10. Centrifuge the 2.0 ml reaction tube at maximum speed for 3 minutes. Transfer the supernatant in a new 2.0 ml reaction tube.
11. Add **400 µl Propan-2-ol** (Isopropanol) to the sample, mix by pipetting up and down several times.

NOTE

It is important that the sample and the Propan-2-ol are mixed vigorously to get a homogeneous solution and avoid the formation of air bubbles.

12. Apply the whole sample to the **Spin Filter** located in a **Receiver Tube**. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 2 minutes.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

13. Discard the Receiver Tube with the filtrate. Place the **Spin Filter** into a new **Receiver Tube**
14. Open the Spin Filter and add **700 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 **Receiver Tube** and **repeat** the **washing step**.

NOTE

After two washing steps, if the **Spin Filter** is still not clean, repeat the washing step one or two times more with **700 µl Washing Solution MS**.

15. Place the **Spin Filter** into a new **Receiver Tube** and centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the **Receiver Tube**
16. Place the Spin Filter into an **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.

10 Troubleshooting

Problem / probable cause	Comments and suggestions
No pellet after enrichment centrifugation step (step 4)	
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.
Enrichment 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.
Spin Filter is not clean after two washing steps	
Depending on the starting material is used	Repeat the washing steps additionally one or two time more

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