



**foodproof**<sup>®</sup>

# **Sample Preparation Kit III**

GMOs, Allergens, Animal ID

## **PRODUCT INSTRUCTIONS**

Documentation for the isolation of DNA from raw material,  
feed and processed food products for direct use in PCR

Product No. KIT230174

**foodproof®**

**Sample Preparation Kit III**  
GMOs, Allergens, Animal ID

Store kit at 15 to 25 °C  
FOR *IN VITRO* USE ONLY

Product No. KIT230174  
50 reactions

**Product Instructions:**  
Revision A, February 2024

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## OVERVIEW

# 1. OVERVIEW

The foodproof® Sample Preparation Kit III is designed for the demanding extraction and purification of DNA from raw materials, animal feed and foodstuffs of plant or animal origin for GMO, allergen or animal identification analysis by real-time PCR. For each of the three options, GMO analysis, allergens detection or animal identification, an optimized protocol is included in this manual.

## 1.1 General Information

### Number of Reactions

The kit is designed for 50 reactions.

### Storage Conditions

Store at 15 to 25 °C.

The components of the foodproof Sample Preparation Kit III are guaranteed to be stable through the expiration date printed on the label.

Improper storage at 2 to 8 °C (refrigerator) or -15 to -25 °C (freezer) will adversely impact DNA purification when precipitates form in the solutions.

After dissolution of Proteinase K, the solution should be aliquoted and stored at -15 to -25 °C. The solution is stable at -15 to -25 °C for 12 months.

## 1.2 Applicability

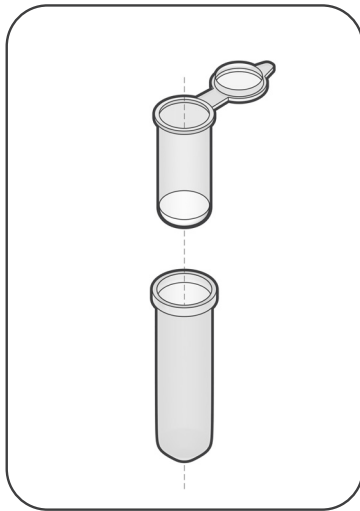
The foodproof Sample Preparation Kit III is optimized for the isolation of DNA from various food samples (raw material, processed food and feed) of plant and animal origin. The quality of the DNA obtained with the kit is highly suitable for qualitative and quantitative applications using any PCR system. The expected yield is 0.1 to 10 µg nucleic acids per 200 mg sample. The obtained yield strongly depends on the processed food type.

The recommended amplicon length for PCR analysis is < 200 bp because the DNA of processed food may be degraded to a certain extent.

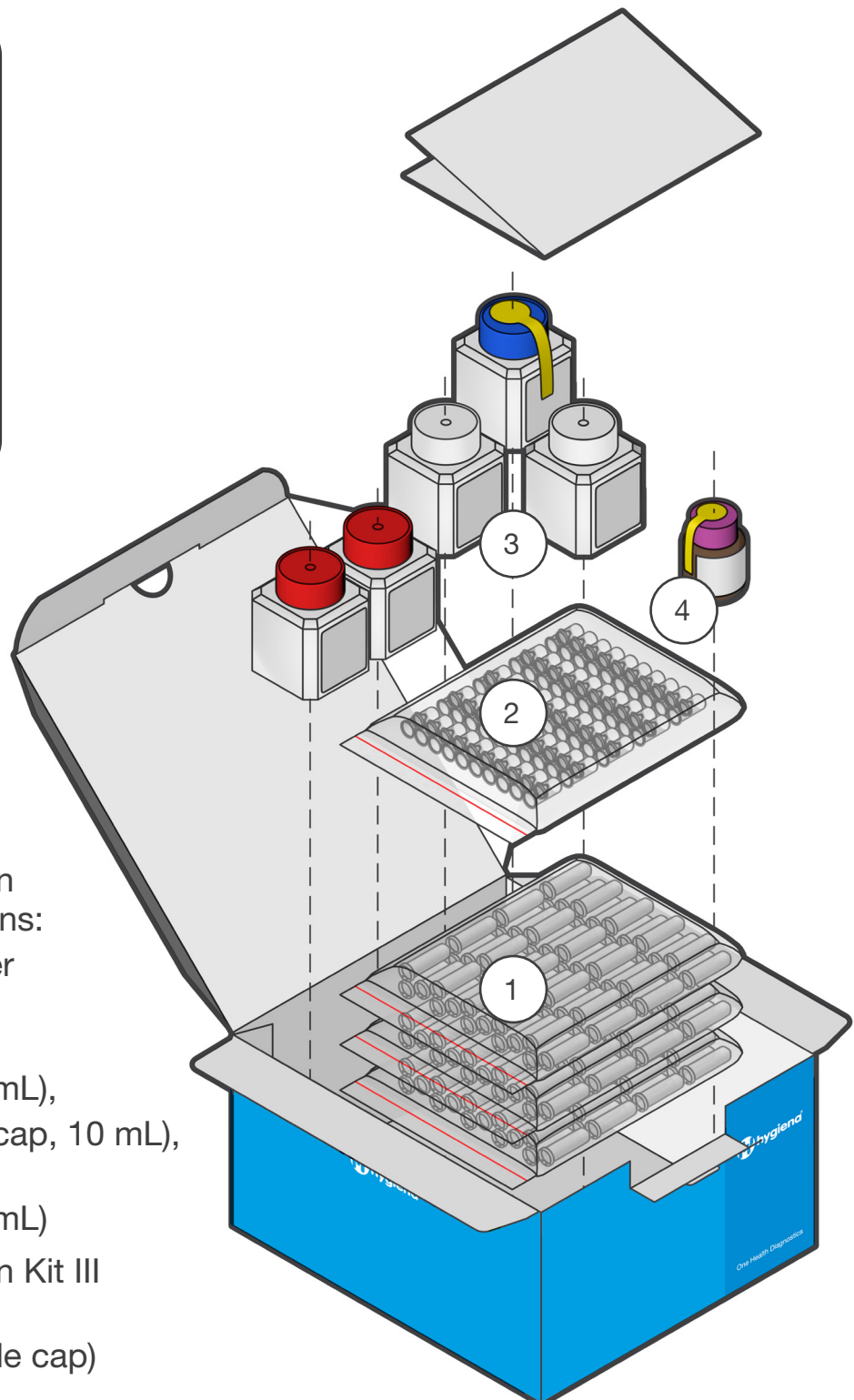
## OVERVIEW

### 1.3 Kit Contents

A schematic representation of the foodproof Sample Preparation Kit III with all its components.



1. 3x bag with 50 collection tubes (2 mL)
2. Bag with 50 filter tubes with two layers of glass fiber fleece
3. Sample Preparation Kit III buffer solutions:  
2x Extraction Buffer (red cap, 45 mL),  
Binding Buffer (colorless cap, 25 mL),  
Wash Buffer (blue cap, 10 mL),  
Elution Buffer (colorless cap, 44 mL)
4. Sample Preparation Kit III additional:  
Proteinase K (purple cap)



## INSTRUCTIONS

# 2. INSTRUCTIONS

This section provides all information for a seamless DNA extraction from a variety of matrices.

## 2.1 Required Material

Most of the required equipment and reagents are available through Hygiena Diagnostics. Please contact us for further information at [www.hygiena.com/support](http://www.hygiena.com/support).



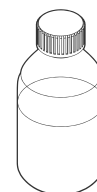
*It is highly recommended only to use the materials described below to guarantee the robustness of the method.*

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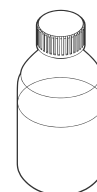
### Reagents

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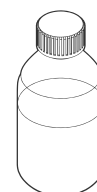
Ethanol, absolute



Isopropanol, absolute



Water, double-distilled



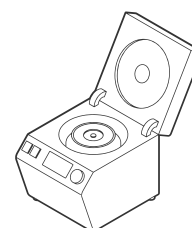
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### Equipment

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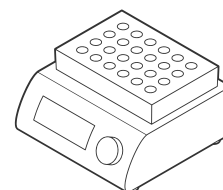
Standard tabletop **microcentrifuge** capable of a 13,000 × g centrifugal force

*e.g., Micro Star 17 - VWR (Order No. D 110 10)*

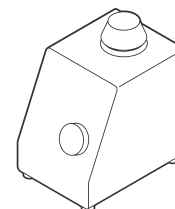


## INSTRUCTIONS

- Heating unit** suitable for 2 mL reaction tubes  
e.g., *AccuBlock™ - Labnet with heating block*



- Vortex mixer**  
e.g., *Vortex Genie - Scientific Industries*



## 2.2 Precautions and Preparations

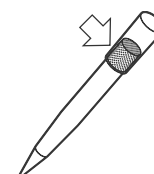
Follow all universal safety precautions governing work with biohazardous materials, e.g., wear lab coats and powder-free gloves at all times. Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

For more information, please refer to the appropriate material safety data sheet (MSDS). The MSDS is available online at [www.hygiena.com](http://www.hygiena.com).

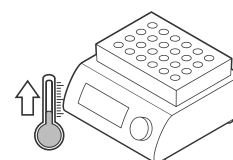


*All solutions except lysis buffer are clear, and should not be used when precipitates have formed. If precipitates have formed, warm the solutions at 15 to 25 °C or in a 37 °C water bath until the precipitates have dissolved.*

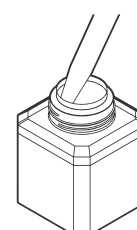
- Always use aerosol-barrier (filter) tips in order to avoid cross-contamination.



- Set the heating unit to 95 °C.

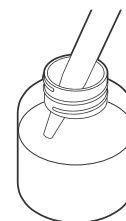


- Prepare Wash Buffer before using the first time.  
Add 40 mL absolute ethanol to Sample Preparation III Wash Buffer, mix well, and store at 15 - 25 °C.  
Label and date bottle after ethanol is added.



## WORKFLOWS

- Prepare Proteinase K before using the first time.  
Dissolve Proteinase K in 5 mL double-distilled water, aliquot solution.  
Store aliquots at  $-15$  to  $-25$  °C, stable for 12 months.



## 2.3 Workflows

The following protocols describe the DNA isolation from 200 mg homogenized sample material. Heterogeneous food samples or unbeaten raw material (e.g., seeds or pellets) must be homogenized in order to obtain a representative sample and to enhance the efficiency of DNA extraction. Suitable methods for homogenization are grinding with a mortar and pestle, or using a homogenizer or bead mill. The most suitable method depends on the sample type analyzed.

After heat lysis and proteinase K digestion, the genomic DNA is bound to the glass fiber filter, washed twice and finally eluted. The purified DNA is free of cellular components and DNA polymerase inhibitors.

Additional aliquots can be prepared and the DNA concentrated by loading them step-by-step into one filter tube. This procedure is recommended if the DNA is degraded due to extensive processing of the food sample during production (e.g., heat, pressure), or if the DNA content of the food sample is low.

## Recommended Kits and Isolation Methods

Analytic Method	Recommended Kits	Recommended DNA Isolation Method
GMO Screening GMO Identification GMO Quantification	foodproof GMO Screening, Identification and Quantification Kits and LyoKits	Procedure A
Allergen Detection Allergen Quantification	foodproof Allergen Detection Kits	Procedure B
Animal Identification	foodproof Animal Detection Kits and LyoKits	Procedure C

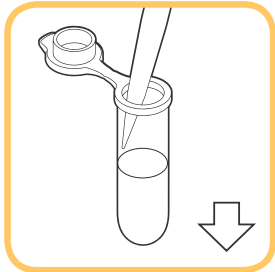


**EXTRACTION PROCEDURE A****2.3.1 EXTRACTION PROCEDURE A: GMO**

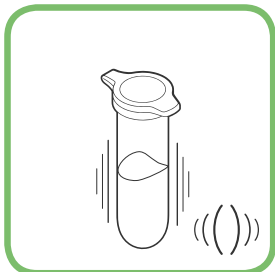
This protocol describes the DNA isolation from 200 mg homogenized sample. The isolated DNA can be used for GMO screening, identification and quantification purposes.

**1. PREPARE SAMPLE**

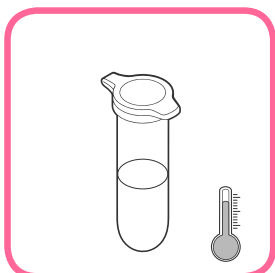
Weigh **200 mg homogenized sample** into a 2 mL reaction tube.

**2. ADD EXTRACTION BUFFER**

Transfer **1,000 µL** extraction buffer (red cap) to homogenized sample.

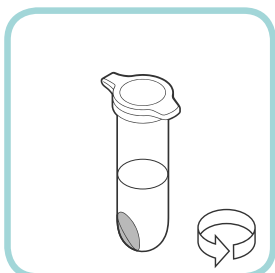
**3. MIX**

**Vortex for 30 sec.**

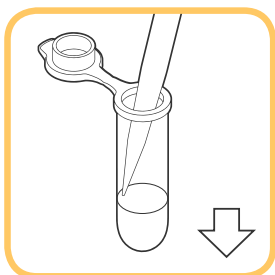
**4. INCUBATE**

**30 min at 80 °C** in a heating unit.

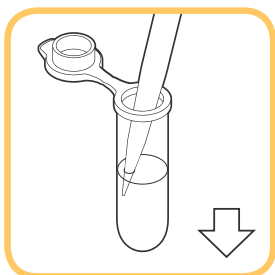
*Note: Mix 2-3 times during the incubation by inverting the tube. If the matrix absorbs the extraction buffer, add additional buffer.*

**5. CENTRIFUGE**

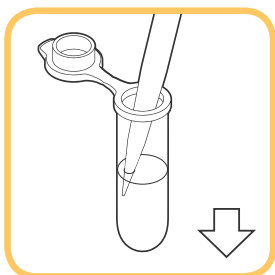
**10 min at 12,000 x g.**

**EXTRACTION PROCEDURE A****6. ADD BINDING BUFFER TO A NEW TUBE**

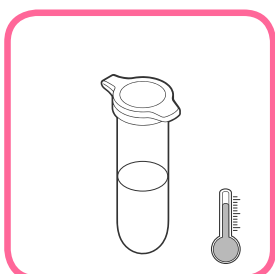
Transfer **400 µL** binding buffer (green cap) to a new 2 mL reaction tube.

**7. ADD SUPERNATANT TO BINDING BUFFER**

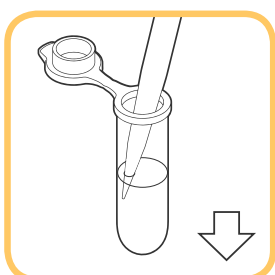
Transfer **600 µL** supernatant (from step 5) to reaction tube with binding buffer (from step 6). Mix well by pipetting up and down.

**8. ADD PROTEINASE K**

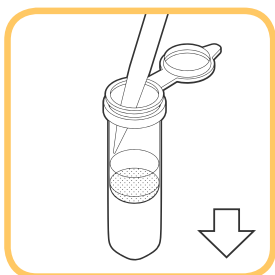
Transfer **80 µL** proteinase K (100 mg / 5 mL ddH<sub>2</sub>O). Mix gently but thoroughly by pipetting up and down.

**9. INCUBATE**

**10 min at 72 °C** in a heating unit.

**10. ADD ISOPROPANOL**

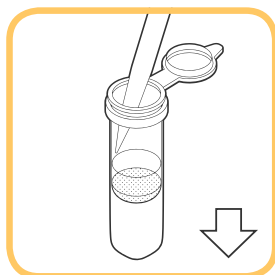
Transfer **200 µL** Isopropanol into each tube. Mix well by pipetting up and down.

**11. FILTER SAMPLE I**

Pipette **650 µL** of mixture into the upper reservoir of a combined filter and collection tube assembly.

**Centrifuge 1 min at 5,000 x g.**

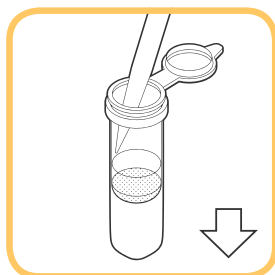
## EXTRACTION PROCEDURE A

**12. FILTER SAMPLE II**

Discard flow-through and collection tube, place filter tube in new collection tube. Apply the remaining mixture to the same filter tube.

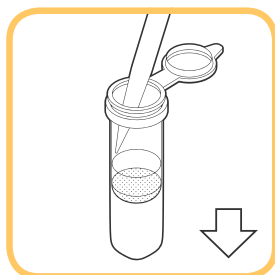
**Centrifuge 1 min at 5,000 x g.**

*Note: If DNA must be pooled, steps 11-12 can be repeated with additional sample preparations.*

**13. WASH SAMPLE I**

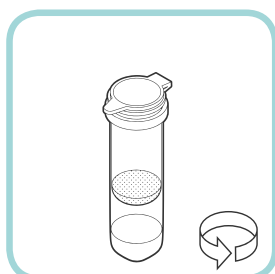
Discard flow-through and collection tube, place filter tube in new collection tube. Add 450  $\mu\text{L}$  **wash buffer (blue cap)** to upper reservoir.

**Centrifuge 1 min at 5,000 x g.**

**14. WASH SAMPLE II**

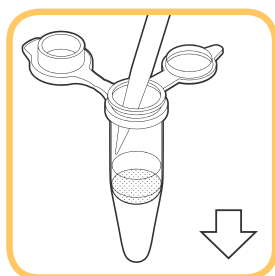
Discard flow-through and reuse collection tube. Add 450  $\mu\text{L}$  **wash buffer (blue cap)** to upper reservoir.

**Centrifuge 1 min at 5,000 x g.**

**15. CENTRIFUGE**

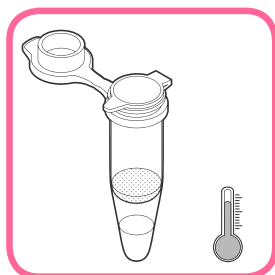
Discard flow-through and reuse collection tube.

Centrifuge to remove residual wash buffer: **10 sec at 13,000 x g.**

**16. ELUTE SAMPLE**

Insert filter tube in a new 1.5 mL reaction tube.

Add **200  $\mu\text{L}$  pre-warmed (70 °C) elution buffer (colorless cap)** onto the glass fleece.

**17. INCUBATE**

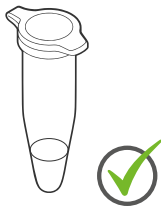
**5 min at room temperature.**

**EXTRACTION PROCEDURE A**



**18. CENTRIFUGE**

**1 min at 5,000 x g.**



**SUPERNATANT FOR DETECTION**

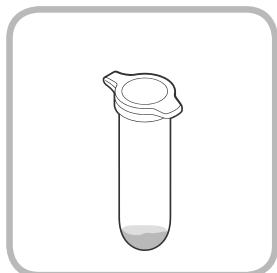
**Use extract for the foodproof GMO PCR (Lyo)kits.**

**For later analysis, store DNA at -15 to -25 °C.**

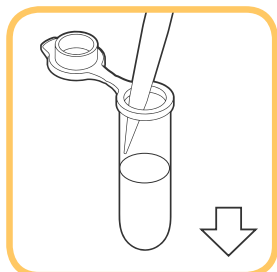
*After thawing, mix by vortexing and briefly centrifuge.*

**EXTRACTION PROCEDURE B****2.3.2 EXTRACTION PROCEDURE B: ALLERGENS**

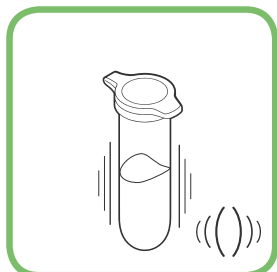
This protocol describes the DNA isolation from a 200 mg homogenized food sample. The isolated DNA can be used for allergen detection and quantification purposes.

**1. PREPARE SAMPLE**

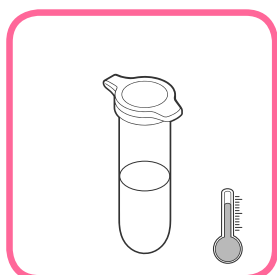
Weigh in **200 mg homogenized sample** in a 2 mL reaction tube.

**2. ADD EXTRACTION BUFFER**

Transfer **1,500 µL** extraction buffer (red cap) to homogenized sample.

**3. MIX**

**Vortex for 30 sec.**

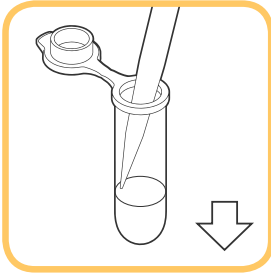
**4. INCUBATE**

**30 min at 80 °C** in a heating unit.

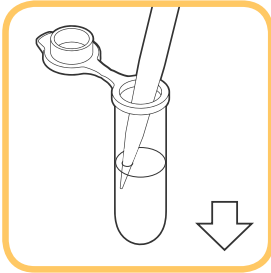
*Note: Mix 2-3 times during the incubation by inverting the tube. If the matrix absorbs the extraction buffer, add additional buffer.*

**5. CENTRIFUGE**

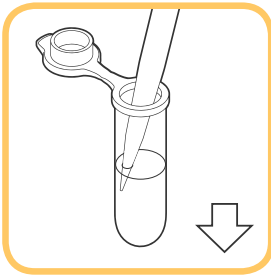
**10 min at 12,000 x g.**

**EXTRACTION PROCEDURE B****6. ADD BINDING BUFFER TO A NEW TUBE**

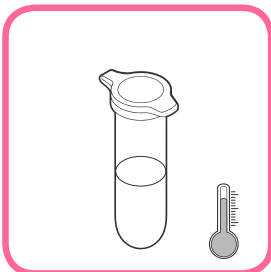
Transfer **400 µL** binding buffer (green cap) to a new 2 mL reaction tube.

**7. ADD SUPERNATANT TO BINDING BUFFER**

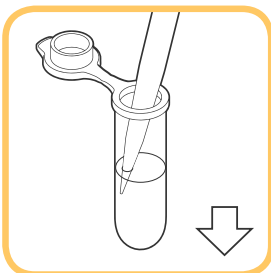
Transfer **600 µL** supernatant (from step 5) to reaction tube with binding buffer (from step 6). Mix well by pipetting up and down.

**8. ADD PROTEINASE K**

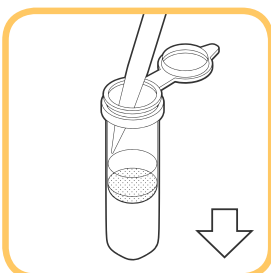
Transfer **80 µL** proteinase K (100 mg / 5 mL ddH<sub>2</sub>O). Mix gently but thoroughly by pipetting up and down.

**9. INCUBATE**

**10 min at 72 °C** in a heating unit.

**10. ADD ISOPROPANOL**

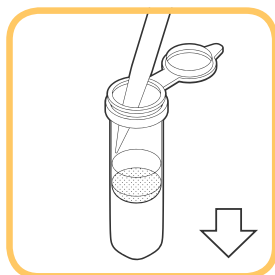
Transfer **200 µL** isopropanol. Mix well by pipetting up and down.

**11. FILTER SAMPLE I**

Pipette **650 µL** of mixture into the upper reservoir of a combined filter and collection tube assembly.

**Centrifuge 1 min at 5,000 x g.**

## EXTRACTION PROCEDURE B

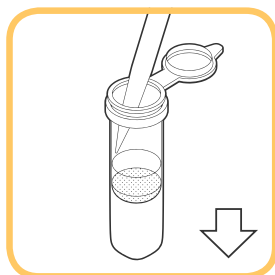


### 12. FILTER SAMPLE II

Discard flow-through and collection tube, place filter tube in new collection tube. Apply the remaining mixture to the same filter tube.

**Centrifuge 1 min at 5,000 x g.**

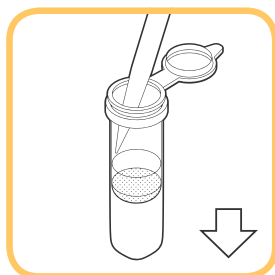
*Note: If DNA must be pooled, steps 11-12 can be repeated with additional sample preparations.*



### 13. WASH SAMPLE I

Discard flow-through and collection tube, place filter tube in new collection tube. Add 450  $\mu\text{L}$  **wash buffer (blue cap)** to upper reservoir.

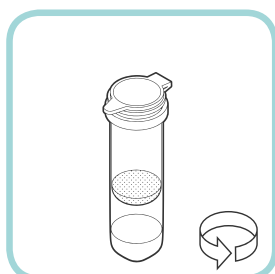
**Centrifuge 1 min at 5,000 x g.**



### 14. WASH SAMPLE II

Discard flow-through and reuse collection tube. Add 450  $\mu\text{L}$  **wash buffer (blue cap)** to upper reservoir.

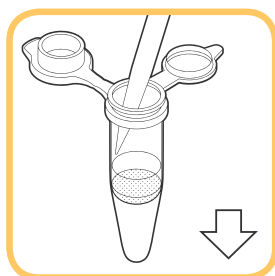
**Centrifuge 1 min at 5,000 x g.**



### 15. CENTRIFUGE

Discard flow-through and reuse collection tube.

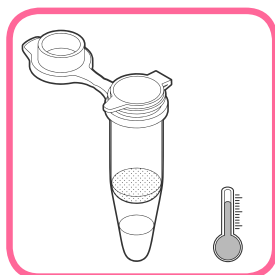
Centrifuge to remove residual wash buffer: **10 sec at 13,000 x g.**



### 16. ELUTE SAMPLE

Insert filter tube in a new 1.5 mL reaction tube.

Add **100  $\mu\text{L}$  pre-warmed (70 °C) elution buffer (colorless cap)** onto the glass fleece.



### 17. INCUBATE

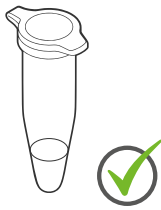
**5 min at room temperature.**

**EXTRACTION PROCEDURE B**



## 18. CENTRIFUGE

1 min at 5,000 x g.



## SUPERNATANT FOR DETECTION

Use extract for the foodproof Allergen PCR (Lyo)kits.

For later analysis later, store DNA at -15 to -25 °C.

*After thawing, mix by vortexing and briefly centrifuge.*

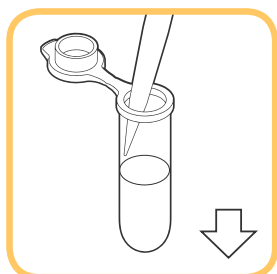


**EXTRACTION PROCEDURE C****2.3.3 EXTRACTION PROCEDURE C: ANIMAL ID**

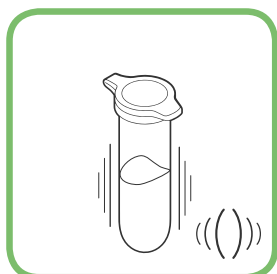
This protocol describes the DNA isolation from 200 mg homogenized food sample. The isolated DNA can be used for animal identification purposes.

**1. PREPARE SAMPLE**

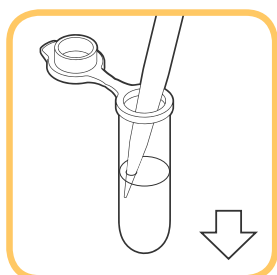
Weigh **200 mg homogenized sample** into a 2 mL reaction tube.

**2. ADD EXTRACTION BUFFER**

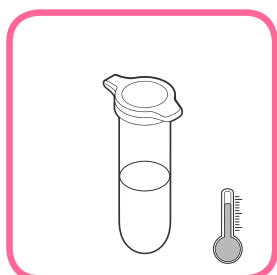
Transfer **1,000 µL** extraction buffer (red cap) to homogenized sample.

**3. MIX**

**Vortex for 30 sec.**

**4. ADD PROTEINASE K**

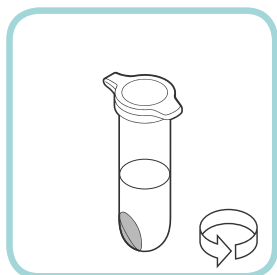
Transfer **80 µL** proteinase K (100 mg / 5 mL ddH<sub>2</sub>O).  
Mix gently but thoroughly by pipetting up and down.

**5. INCUBATE**

**30 min at 72 °C** in a heating unit.

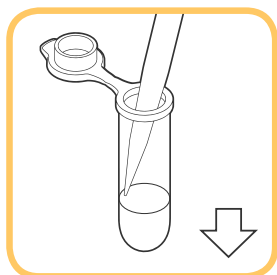
*Note: Mix 2-3 times during the incubation by inverting the tube. If the matrix absorbs the extraction buffer, add additional buffer.*

## EXTRACTION PROCEDURE C



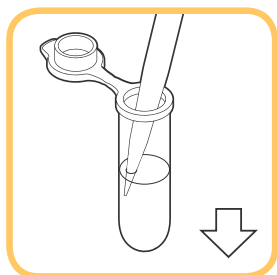
### 6. CENTRIFUGE

10 min at 12,000 x g.



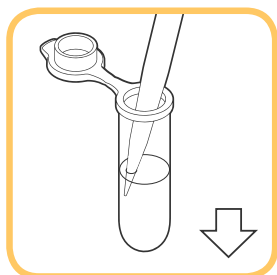
### 7. ADD BINDING BUFFER TO A NEW TUBE

Transfer **400 µL** binding buffer (green cap) to a new 2 mL reaction tube.



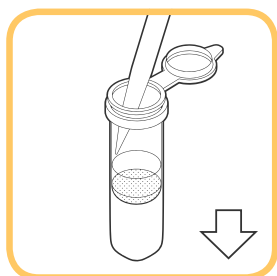
### 8. ADD ISOPROPANOL

Transfer **200 µL** isopropanol.  
Mix well by pipetting up and down.



### 9. ADD SUPERNATANT TO BINDING BUFFER

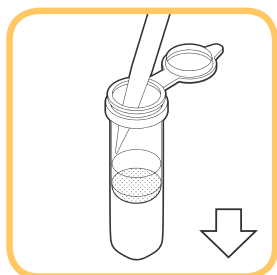
Transfer **600 µL** supernatant (from step 5) to reaction tube with binding buffer and isopropanol (from step 7). Mix well by pipetting up and down.



### 10. FILTER SAMPLE I

Pipette **650 µL** of mixture into the upper reservoir of a combined filter and collection tube assembly.

**Centrifuge 1 min at 5,000 x g.**



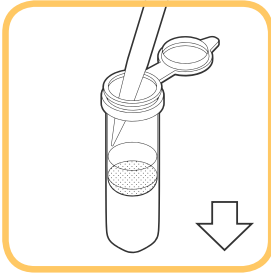
### 11. FILTER SAMPLE II

Discard flow-through and collection tube, place filter tube in new collection tube. Apply the remaining mixture to the same filter tube.

**Centrifuge 1 min at 5,000 x g.**

*Note: If DNA must be pooled, steps 11-12 can be repeated with additional sample preparations.*

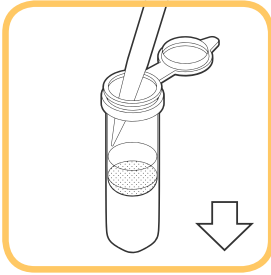
## EXTRACTION PROCEDURE C



### 12. WASH SAMPLE I

Discard flow-through and collection tube, place filter tube in new collection tube.  
Add 450  $\mu\text{L}$  **wash buffer (blue cap)** to upper reservoir.

**Centrifuge 1 min at 5,000 x g.**



### 13. WASH SAMPLE II

Discard flow-through and reuse collection tube.  
Add 450  $\mu\text{L}$  **wash buffer (blue cap)** to upper reservoir.

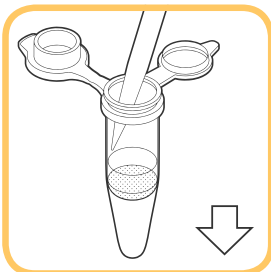
**Centrifuge 1 min at 5,000 x g.**



### 14. CENTRIFUGE

Discard flow-through and reuse collection tube.

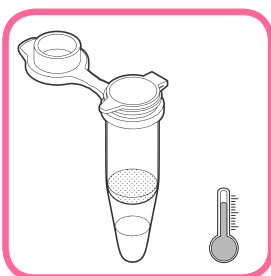
Centrifuge to remove residual wash buffer: **10 sec at 13,000 x g.**



### 15. ELUTE SAMPLE

Insert filter tube in a new 1.5 mL reaction tube.

Add **200  $\mu\text{L}$  pre-warmed (70 °C) elution buffer (colorless cap)** onto the glass fleece.



### 16. INCUBATE

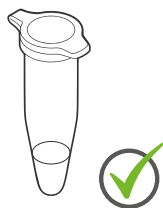
**5 min at room temperature.**



### 17. CENTRIFUGE

**1 min at 5,000 x g.**

## EXTRACTION PROCEDURE C



### **SUPERNATANT FOR DETECTION**

**Use extract for the foodproof Animal ID PCR (Lyo)kits.**

**For later analysis, store DNA at -15 to -25 °C.**

*After thawing, mix by vortexing and briefly centrifuge.*

## TROUBLESHOOTING

## 2.4 Troubleshooting

Problem	Possible Cause	Recommendation
Extract inhibits PCR	DNA extract contains too many PCR inhibitors.	Dilute DNA extract, e.g., 1:10, or reduce the amount of extracted DNA, e.g., for LyoKits, use 5 $\mu$ L instead of 25 $\mu$ L.
Low DNA yield or purity	Improper storage of kit components.	Store kit reagents at 15 to 25°C. Close all reagent bottles tightly after each use to preserve pH and stability, and to prevent contamination.
		After any lyophilized reagent is reconstituted, aliquot it, then store the aliquots at -15 to -25 °C.
	Precipitates have formed.	If precipitates have formed, warm the solutions at 15 to 25 °C or in a 37 °C water bath until the precipitates have dissolved.
	Ethanol not added to wash buffer.	Prepare wash buffer as described in Section 2.2.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
	Homogenization of food sample not sufficient.	Use a mortar and pestle or a commercial product, such like mixer or bead mills for disruption/homogenization.
Suboptimal reaction conditions.		Ensure proper disruption and heating conditions.
		Verify correct temperature of the heating block with a thermometer.
Absorbency ( $A_{260}$ ) reading of product too high	Glass fibers which can co-elute with DNA, scatter light.	After elution step is complete, remove filter from tube containing eluted sample, spin sample tube for 2 min at maximum speed. Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Sample “pops” out of wells in agarose gels	Eluate containing the purified DNA is contaminated with ethanol from the wash buffer.	After the last wash step, ensure the flow-through containing wash buffer does not contact the bottom of the filter tube. If this has occurred, empty collection tube, reinsert the contaminated filter tube, and centrifuge for 30 sec.

## SUPPORT

### 2.5 Support

If you have questions or experience any problems with our products, please contact us:



[www.hygiena.com/support](http://www.hygiena.com/support)

Our aim is to provide you with a solution as quickly and effectively as possible. We would also like you to contact us if you have any suggestions for improving the product or in case you would like to use our product for a different application. We highly value your feedback.

## **3. ADDITIONAL INFORMATION**

### **3.1 General Information**

#### **Quality Control**

All products are regularly monitored by our quality control. You can find the certificate of analysis (COA) on our website. If you would like to carry out your own quality control, you will find the analysis method described in the certificate.

#### **Waste Disposal**

All contaminated and potentially infectious material, like enrichment cultures or food samples, should be autoclaved before disposal and eliminated according to local rules and regulations. For proper disposal of unused chemicals, please refer to the SDS.

#### **Warranty and Disclaimer of Liability**

“Limited Warranty” and “Disclaimer of Liability”: Hygiena Diagnostics GmbH warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

- (1) The product is used according to the guidelines and instructions set forth in the product literature;
- (2) Hygiena Diagnostics GmbH does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by Hygiena Diagnostics GmbH; defects caused by misuse or use contrary to the instructions supplied, or improper storage or handling of the product;
- (3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty;
- (4) Hygiena Diagnostics GmbH does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of Hygiena Diagnostics GmbH;
- (5) Hygiena Diagnostics GmbH does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;
- (6) Hygiena Diagnostics GmbH reserves the right to replace or allow credit for any modules returned under this warranty.

## TABLE OF CONTENTS

### 3.2 Trademarks

**foodproof**<sup>®</sup>, **microproof**<sup>®</sup>, **vetproof**<sup>®</sup>, ShortPrep<sup>®</sup>, StarPrep<sup>®</sup>, RoboPrep<sup>®</sup> and LyoKit<sup>®</sup> are registered trademarks of Hygiena Diagnostics GmbH.

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Other brand or product names are trademarks of their respective holders.

### 3.3 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S400 06.1

### 3.4 Change Index

*Version 1, June 2015:*

First version of the product insert.

*Version 2, September 2020:*

New document layout and content.

*Revision A, February 2024:*

Rebranding and new layout.

S 400 06.1 20 -> INS-KIT230174-RevA



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