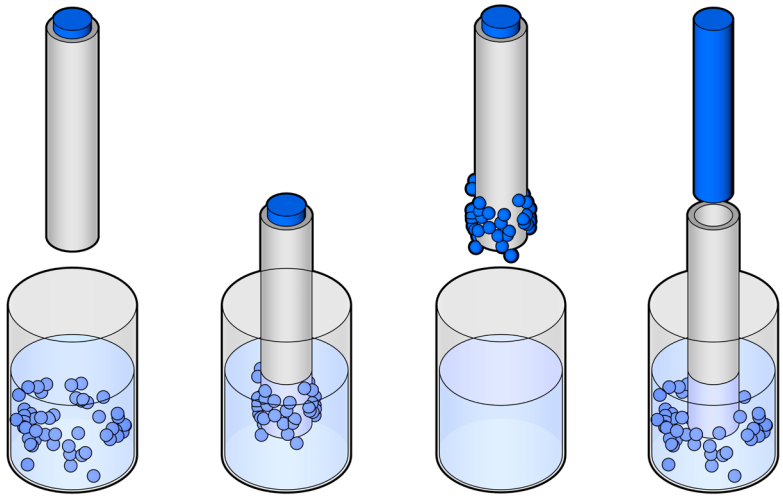

foodproof®

Magnetic Preparation Kit III

Automated DNA extraction from plant and animal origin

PRODUCT INSTRUCTIONS

Documentation for automated isolation of DNA from raw material and food products of plant and animal origin using the foodproof RoboPrep® 32 or the KingFisher™ Flex instrument.

Product No. KIT230182

foodproof®

Magnetic Preparation Kit III

Automated DNA extraction from plant and animal origin

Product No. KIT230182

Kit for 480 reactions

Store Box A and B at 15 to 25 °C

FOR *IN VITRO* USE AND RESEARCH USE ONLY

PRODUCT INSTRUCTIONS

Revision A, January 2024

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1. OVERVIEW

The foodproof® Magnetic Preparation Kit III is designed for the rapid and safe high-throughput extraction of DNA from up to 200 mg sample material and food products from plant and animal origin. The kit uses Proteinase K for the lysis, and magnetic beads for the washing and purification steps, resulting in a high yield of highly purified DNA. After preparing and loading the plates into the foodproof RoboPrep® 32 or the KingFisher™ Flex instrument, all remaining purification steps are performed automatically without the need for external centrifugation steps. Each component of the kit has been tested against predetermined specifications to ensure consistent product quality.

1.1 General Information

Number of Reactions

The kit is designed for 480 reactions.

Storage Conditions

All buffers and kit components of the foodproof Magnetic Preparation Kit III should be stored at 15 to 25 °C and are stable for at least 12 months under these conditions.

Proteinase K: Lyophilized Proteinase K should be stored at 15 to 25 °C.
Reconstituted Proteinase K must be stored at -20 °C. Avoid multiple freeze-thaw cycles of diluted Proteinase K.

Magnetic Beads: The magnetic particles should be stored at 15 to 25 °C.

Wash and Binding Buffers: Buffers charged with 2-propanol should be stored at room temperature and must be sealed accordingly. If any precipitates are visible within the provided solutions, dissolve them by carefully heating up to 30 °C.

1.2 Test Principle

The foodproof Magnetic Preparation Kit III in combination with the foodproof RoboPrep 32 or the KingFisher Flex instrument provides fully automated purification of total genomic DNA from up to 200 mg of raw material and food products of plant and animal origin. In combination with additional Lysis Buffer and Proteinase K, a protocol for up to 2 g of sample material is available (contact us for information). The DNA isolation process is based on patented magnetic bead technology, which relies on the interaction of nucleic acids with coated magnetic particles under suitable buffer conditions. The kit provides high-quality DNA, which can be used directly in PCR applications.

The foodproof RoboPrep 32 and the KingFisher Flex instrument perform all steps of the DNA purification procedure automatically. The procedure requires only minimal user

interaction, namely the initial loading of the system and the preparation of the sample material. Sample cross-contamination and reagent crossover is effectively eliminated by the provided purification assay.

Both instruments use magnetic rods to transport the DNA, bound to magnetic particles, through the various purification phases: binding, washing and elution. The volume of buffers and other liquids necessary for DNA isolation is reduced to a minimum. Eliminating the direct liquid handling and increasing the automation level results in a fast, reliable and robust technique.

To achieve effective lysis and high DNA yields, the samples are first lysed with optimized lysis buffer and Proteinase K in a separate step. After lysis, the DNA binds to the magnetic beads whereas contaminants, metabolites and enzyme inhibitors are efficiently removed during the following three wash steps. Finally, highly purified DNA is eluted in elution buffer. The purified, high-quality DNA is ready for use in downstream applications such as PCR or can be stored at -20 °C for later use.

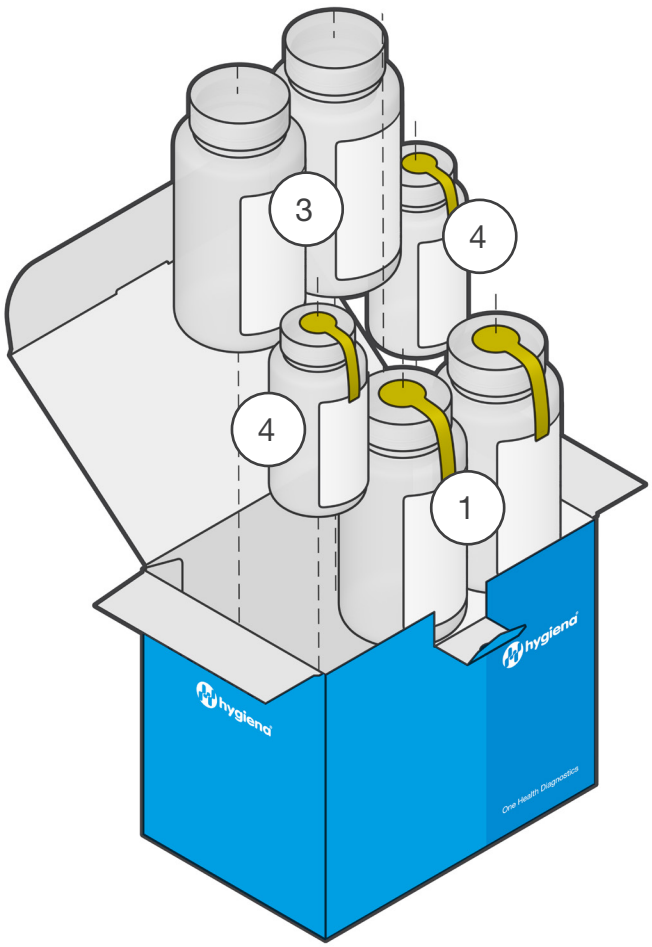
Basic Steps

Step	Description
1	Sample lysis by incubation with the foodproof Magnetic Preparation Kit III Lysis Buffer and foodproof Magnetic Preparation Kit III Proteinase K
2	DNA is bound to magnetic beads
3	Washing of bound DNA to remove proteins and other cellular impurities
4	Purified DNA is recovered using the elution buffer

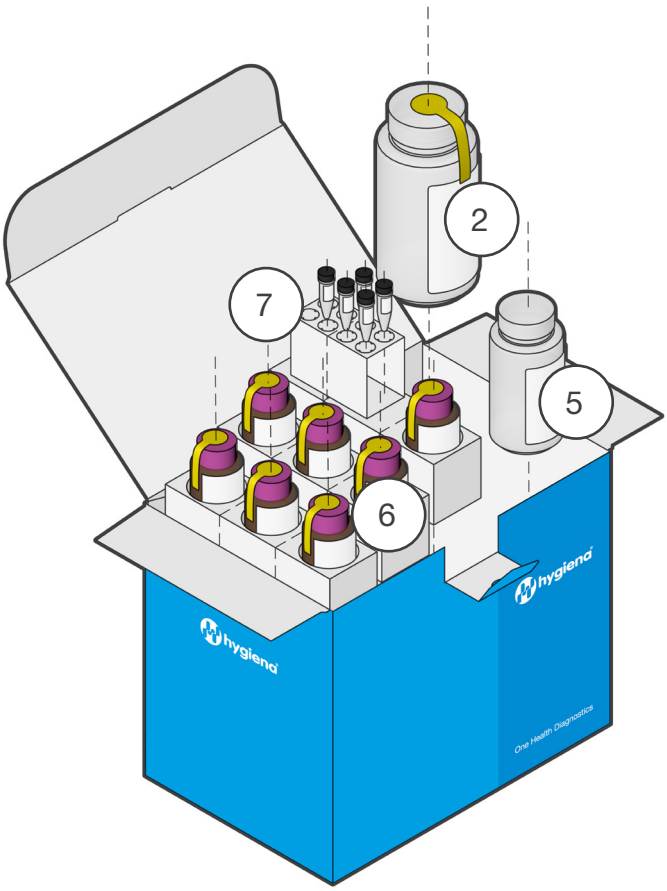
1.3. Kit Contents

This a schematic representation of the foodproof Magnetic Preparation Kit III with all its components.

BOX A



BOX B



1. 2x Lysis Buffer (2x 400 mL)
2. Binding Buffer (30 mL)
3. 2x Wash Buffer I (2 x 160 mL)
4. 2x Wash Buffer II (2 x 45 mL)
5. Elution Buffer (110 mL)
6. 7x Proteinase K (7 x 100 mg)
7. 5x Magnetic Beads (5 x 0.85 mL)

2. INSTRUCTIONS

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

2.1 Required Material

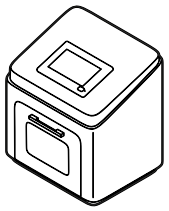
Most of the required equipment and reagents are available through Hygiena®.
Please contact us for further information.

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

Equipment

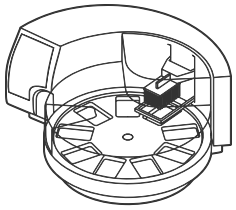
☐ **foodproof RoboPrep® 32,** capable of 32 samples per run
Product no. MCH230000

Only for Automated Extraction Procedure 2.3.1

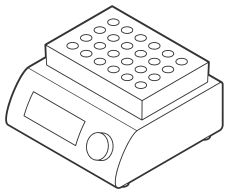


☐ **KingFisher™ Flex,** capable of 96 samples per run

Only for Automated Extraction Procedure 2.3.2



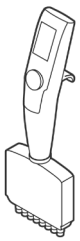
☐ **Thermoshaker** suitable for 2 mL tubes
e.g., Eppendorf - ThermoMixer® comfort



Recommended:

☐ **Multichannel pipette** and filter tips
e.g., 8-Channel Pipette VIAFLO - INTEGRA Biosciences
with GripTips: 50 to 1,250 µL

or EP Xplorer Plus Electronic Multichannel Pipette
with Filter tips: 50 to 1,250 µL

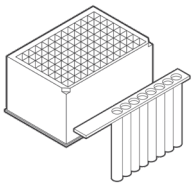


Consumables

☐ **MPK III Consumable List for foodproof RoboPrep® 32**

Deep-well plates (2 mL),
Tip combs

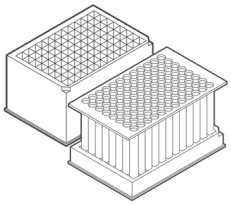
Only for Automated Extraction Procedure 2.3.1



☐ **MPK III Consumable List for KingFisher™ Flex**

Deep-well plates (2 mL),
Elution plate (0.2 mL),
Tip combs,
Sealing foil (for 96 microplates)

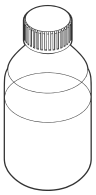
Only for Automated Extraction Procedure 2.3.2



Reagents

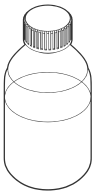
☐ **2-Propanol, absolute**

Not provided by Hygiena Diagnostics GmbH



☐ **Water, double-distilled**

Not provided by Hygiena Diagnostics GmbH

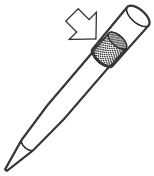


2.2 Precautions and Preparations

Follow all universal safety precautions governing work with biohazardous materials, e.g., wear lab coats and 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 (SDS). The SDS is available online at www.hygiena.com/sds.

☐ Always use filter tips in order to avoid cross-contamination.



☐ Prepare **Proteinase K** before using it the first time (calculate the required number of bottles). Dissolve Proteinase K in **5 mL double-distilled water**, aliquot solution.

Store aliquots at –15 to –25 °C, stable for 12 months.

☐ Prepare **Binding Buffer** before using it the first time.

Add 120 mL 2-Propanol, absolute, mix well, and store at 15 to 25 °C.

Label and date bottle after 2-Propanol has been added and tick off the corresponding box on the label.

☐ Prepare **Wash Buffer I** before using it the first time.

Add 160 mL 2-Propanol, absolute, mix well, and store at 15 to 25 °C.

Label and date bottle after 2-Propanol has been added and tick off the corresponding box on the label.

☐ Prepare **Wash Buffer II** before using it the first time.

Add 180 mL 2-Propanol, absolute, mix well, and store at 15 to 25 °C.

Label and date bottle after 2-Propanol has been added and tick off the corresponding box on the label.

2.3 Workflows

The following procedures describe the automated DNA extraction from plant and animal species with the foodproof Magnetic Preparation Kit III in combination with our two extraction devices:

The foodproof RoboPrep 32 for low to medium throughput (32 samples per run) and the KingFisher Flex instrument for medium to high throughput (96 samples per run).

After preparing and loading the plates into the devices, no further manual steps are necessary. A complete run takes approximately 35 minutes.

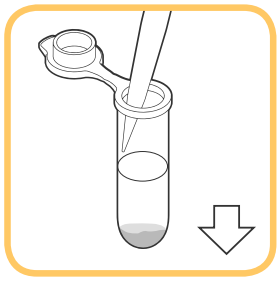
2.3.1 Extraction Procedure A: RoboPrep® 32

This protocol describes semi-automated DNA extraction from 200 mg sample using the foodproof RoboPrep 32. It consists of two parts: The manual preparation of samples and plates and the automated extraction run.

Please ensure that you have prepared all required reagents (see 2.2 Precautions and Preparations).

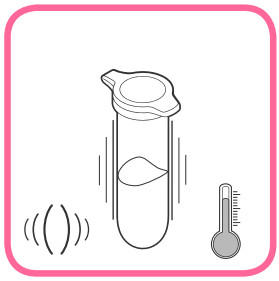
For a 16-sample run you will need the following consumables: 1 deep-well plate, 2 tip combs.

The RoboPrep® 32 processes two deep-well plates simultaneously (for a total of 32 samples).



1. ADD SAMPLE

Transfer up to **200 mg homogenized sample material** into a 2 mL reaction tube and add **1,400 µL of Lysis Buffer** and **70 µL of Proteinase K**.

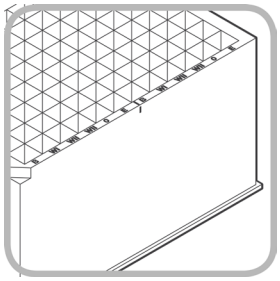


2. INCUBATE

30 min at 65 °C with shaking in the heating unit.

3. CENTRIFUGE

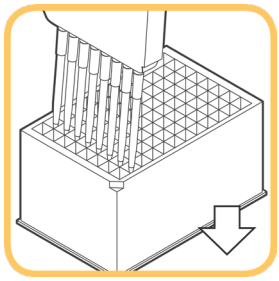
10 min at 12,000 x g.



4. MARK PLATE ROWS

Label the rows of the deep-well plate for the individual buffers to prevent pipetting errors when preparing the deep-well plate.

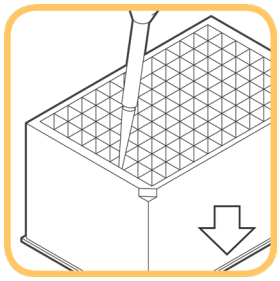
Note: As a naming system, you can use the following abbreviations for buffers: B: Binding Buffer, WI: Wash Buffer I, WII: Wash Buffer II, 0: No buffer, E: Elution Buffer.



5. ADD BUFFER TO RESPECTIVE ROWS

Row 1 (and 7): add **250 µL Binding Buffer** and **8 µL Magnetic Beads** per well.
Row 2 (and 8): add **600 µL Wash Buffer I** per well.
Row 3 (and 9): add **600 µL Wash Buffer I** per well.
Row 4 (and 10): add **800 µL Wash Buffer II** per well.
Row 5 (and 11): remains empty.
Row 6 (and 12): add **200 µL Elution Buffer** per well.

Note: Before pipetting, take care that the magnetic beads are homogenously distributed. We recommend using multichannel pipettes to speed up liquid handling.



6. ADD SAMPLES TO DEEP-WELL PLATE

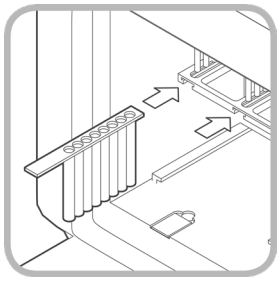
Transfer **500 µL** lysate to row 1 (and 7) of the deep-well plate.

Note: Avoid cross-contamination by NOT pipetting up and down during this step.

7. SELECT PROGRAM

Start the foodproof RoboPrep 32 instrument.

Select the pre-installed program '**MPK III**' via touchscreen.

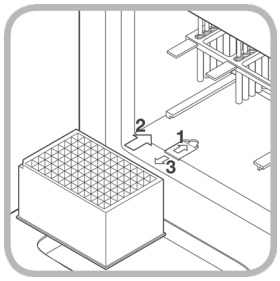


8. INSTALL TIP COMBS

Open the front door.

Insert tip combs.

Note: Number of tip comps (tc) depends on number of samples that are processed: samples ≤ 8: 1 tc; samples 9-16: 2 tc; samples 17-24: 3 tc; samples 25-32: 4 tc.

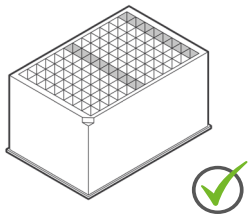


9. INSERT PLATE(S) AND START RUN

To enter the deep-well plate, lower the heating block by pushing the lever backwards (1), slide in the plate (2) and push the lever in the opposite direction to raise the heating block and to lock the plate in place (3).

Start the run. All the extraction steps will run automatically.

Note: Details about the automated extraction steps and programming of the instrument can be found in the appendix.



READY FOR DETECTION

Rows 6 (and 12) of the plate contain the extracted DNA from the samples.

RECOMMENDATION: Use eluted DNA right after extraction. Additionally, it is recommended to analyze a 1:4 dilution, because the eluate may still contain PCR-inhibiting substances.

For later analysis, transfer extracts to tubes and store at -15 to -25 °C. For long-term storage, keep at -80 °C. After thawing, mix briefly by vortexing and centrifuge at high speed for 1 min.

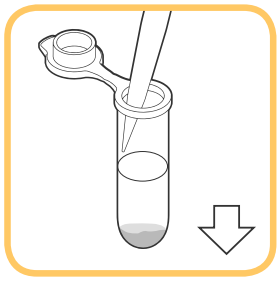
Note: If the elution plate still visibly contains magnetic beads, you may centrifuge it to avoid interference with the detection kit (1 min at high speed). The DNA is in the supernatant.

2.3.2 Extraction Procedure B: KingFisher Flex

This protocol describes the automated DNA extraction from up to 200 mg sample using the KingFisher Flex instrument. It consists of two parts: The manual preparation of samples and plates and the automated extraction run.

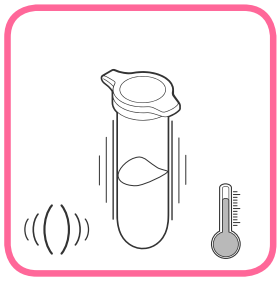
Please make sure that you have prepared all required reagents (see 2.2 Precautions and Preparations).

For a 96-sample run you will need the following consumables:
4 deep-well plates, 2 elution plates, 1 tip comb.



1. ADD SAMPLE

Transfer up to **200 mg homogenized sample material** into a 2 mL reaction tube and add **1,400 µL of Lysis Buffer** and **70 µL of Proteinase K**.

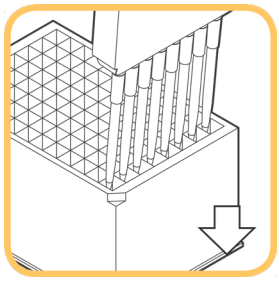


2. INCUBATE

30 min at 65 °C with shaking in the heating unit.

3. CENTRIFUGE

10 min at 12,000 x g.

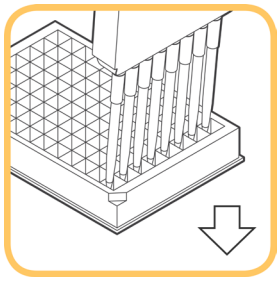


4. PREPARE WASHING AND BINDING PLATES

Fill the deep-well plates with wash buffer and label them:

WASH PLATE 1: add **600 µL Wash Buffer I** per well.
WASH PLATE 2: add **600 µL Wash Buffer I** per well.
WASH PLATE 3: add **800 µL Wash Buffer II** per well.
BINDING PLATE: add **250 µL Binding Buffer** and **8 µL Magnetic Beads** per well.

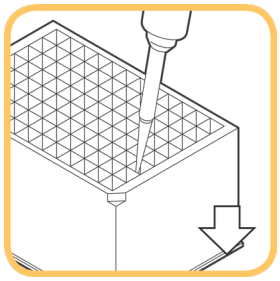
Note: Before pipetting, take care that the magnetic beads are homogenously distributed. We recommend using multichannel pipettes to speed up liquid handling.



5. PREPARE ELUTION PLATE

Add **200 µL Elution Buffer** to every well of the elution plate.

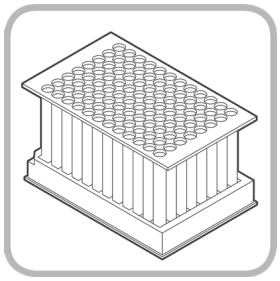
Note: We recommend using multichannel pipettes to speed up liquid handling.



6. ADD SAMPLES TO BINDING PLATE

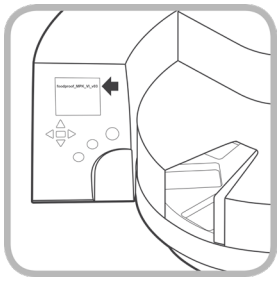
Transfer **500 µL** lysate to the binding plate.

Note: Avoid cross-contamination by NOT pipetting up and down during this step.



7. PREPARE TIP PLATE

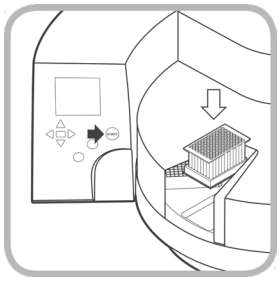
Place the Tip Comb 96 DWH (Deep Well Holder) on a Tip Plate.
Use one of the provided Elution Plates as a Tip Plate (these are identical).



8. SELECT PROGRAM

Start the KingFisher Flex instrument.

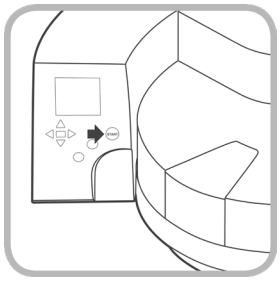
Open folder 'DNA' and select the pre-installed program '**foodproof_MPK_III**'.



9. LOAD INSTRUMENT WITH PLATES

The program tells you which plate you have to put into the instrument next.
Load the instrument with the requested plate and
press 'Start' to load the next plate.

Note: After pressing 'Start' the rotary plate will automatically move into the correct loading position.

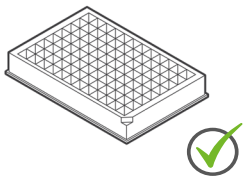


10. START RUN

Once all the plates have been placed in the instrument, confirm again by pressing 'Start'.

All the extraction steps will run automatically.

Note: Details about the automated extraction steps and programming of the instrument can be found in the appendix.



READY FOR DETECTION

The elution plate contains the extracted DNA from the samples.

RECOMMENDATION: Use eluted DNA right after extraction. Additionally, it is recommended to analyze a 1:4 dilution, because the eluate may still contain PCR-inhibiting substances.

For later analysis, transfer extracts to tubes and store at -15 to -25 °C. For long-term storage, store at -80 °C. After thawing, mix briefly by vortexing and centrifuge at high speed for 1 min.

Note: If the elution plate still visibly contains magnetic beads, you may centrifuge it to avoid interference with the detection kit (1 min at high speed). DNA is in the supernatant.

2.4 Troubleshooting

Problem	Possible Cause	Recommendation
Low DNA yield or purity	Kit stored under non optimal conditions	Store diluted Proteinase K at -20 °C Store all other kit components at room temperature
	Buffer or other reagents were exposed to conditions that reduced their effectiveness	Store all buffers at room temperature Close all reagent bottles tightly after each use to preserve pH and stability, and to prevent contamination After lyophilized reagents are reconstituted, store at -20 °C
	2-Propanol not added to Wash Buffer I or Wash Buffer II	Add absolute 2-Propanol to the Wash Buffer I and Wash Buffer II before using After adding 2-Propanol, mix the Wash Buffer I and Wash Buffer II well, and store at room temperature Always mark the Wash Buffer I and Wash Buffer II bottle to indicate the addition of 2-Propanol
	Low amount of Magnetic Beads	Mix the Magnetic Beads thoroughly before pipetting to the Binding Plate
	Suboptimal reaction conditions	Don‘t forget to install tip combs Ensure proper heating conditions Ensure correct positioning of heating blocks in foodproof RoboPrep 32 and KingFisher Flex instrument Verify correct temperature of the heating block with a thermometer
DNA does not perform well in real-time PCR	Salt carryover during elution	Check the Wash Buffers for salt precipitates. If there are any precipitates, dissolve these precipitates by careful warming Ensure that Wash Buffers are stored at room temperature
Low A260:A280 ratio from UV measurement, eluted DNA is brown-colored	Small part of the magnetic particles are left in the elution	Centrifuge at full speed for 1 min and transfer supernatant (contains DNA) to a new tube

2.6 Support

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

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.

3.2 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 13.2 L

3.3 Change Index

Revision A, January 2024:
Rebranding and new layout.
S 400 13.2 L 20 -> INS-KIT230182-REVA

4. APPENDIX

4.1 foodproof RoboPrep 32

Details for automated purification steps:

Binding of the DNA: Automatic sample mixing for 5 min. Magnetic beads separation. Transfer of the magnetic particles to Wash Buffer I.

First Wash: Automatic sample mixing for 3 min. Magnetic beads separation. Transfer of the magnetic particles to Wash Buffer II.

Second Wash: Automatically sample mixing for 3 min. Magnetic beads separation. Transfer of the magnetic particles to Wash Buffer II.

Third Wash: Automatic sample mixing for 3 min. Magnetic beads separation. Transfer of the magnetic beads to the Elution Buffer.

Elution of the DNA: Incubation of magnetic particles in the Elution Buffer for 10 minutes by continuously mixing. Magnetic beads separation. The magnetic beads will automatically be removed and transferred in Wash Buffer III (disposal).

Self-programming of the instrument

Edit and run the experiment program as follows:

RUN	Well No. (0-6)	Name	Standby (0-30 min)	Mix (1-30 min)	Volume (100-1000 µl)	Mix Speed (1-3)	Mag (0-120 sec)	Temp (40-80 °C)	Pause
☒	1	Binding	0	5	758	3	30	0	☐
☒	1	Binding	0	0	758	0	60	0	☐
☒	2	Wash-1	0	3	600	3	30	0	☐
☒	2	Wash-1	0	0	600	0	30	0	☐
☒	3	Wash-2	0	3	600	3	30	0	☐
☒	3	Wash-2	0	0	600	0	30	0	☐
☒	4	Wash-3	0	3	800	3	30	0	☐
☒	4	Wash-3	0	0	800	0	30	0	☐
☒	6	Elution	7	10	200	1	120	80	☐
☒	4	Waste	0	1	800	3	0	0	☐

4.2 KingFisher Flex

Details for automated purification steps:

Binding of the DNA: Automatic sample mixing for 5 min. Magnetic Beads separation. Transfer of the magnetic particles to Washing Plate I.

First Wash: Automatic sample mixing for 1.5 min. Magnetic Beads separation. Transfer of the magnetic particles to Washing Plate II.

Second Wash: Automatic sample mixing for 1 min. Magnetic Beads separation. Transfer of the magnetic particles to Washing Plate III.

Third Wash: Automatic sample mixing for 1 min. Magnetic Beads separation.

Drying: Drying of the Magnetic Beads outside Washing Plate III for 5 minutes. Transfer of the Magnetic Beads to the Elution Plate.

Elution of the DNA: Incubation of magnetic particles in the Elution Buffer for 10 minutes at 90 °C by continuously mixing. Magnetic Beads separation. The Magnetic Beads will automatically be removed and transferred in Washing Plate III (disposal).

Self-programming of the instrument:

Protocol information

Protocol name: foodproof_MPK_III
Kit name: foodproof MPK III
Description: KingFisher™ Flex protocol for isolation of genomic DNA from raw material and food products of plant and animal origin.

Plate Layouts

Binding Plate		Microtiter 96 Deep-Well Plate	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
Sample	500	-	Sample
Binding Buffer	250	-	Reagent
Magnetic Beads	8	-	Reagent
Washing Plate I		**Microtiter 96 Deep-Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
Wash Buffer I	600	-	Reagent
Washing Plate II		**Microtiter 96 Deep-Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
Wash Buffer I	600	-	Reagent
Washing Plate III		**Microtiter 96 Deep-Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
Wash Buffer II	800	-	Reagent
Elution Plate		**Microtiter 96 Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
Elution Buffer	200	-	Reagent
Elution Plate		**Microtiter 96 Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
-	-	-	-

File Steps

Tip 1		**Tip Comb 96 DWH**	
	Pick-Up	**Tip Plate**	
	Binding	**Binding Plate**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	10
	Washing 1	**Washing Plate I**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:30, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	3
	Washing 2	**Washing Plate II**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	3
	Washing 3	**Washing Plate III**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	3
	Dry1	**Washing Plate III**	
		Dry time	00:05:00
		Tip position	Outside well / tube
	Eluting	**Elution Plate**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	90
		Preheat	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	3
	ReleaseBeads1	**Washing Plate III**	
		Release time, speed	00:00:10, Fast
	Leave	**Tip Plate**	

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