foodproof® Magnetic Preparation Kit IV

Revision B, November 2024

For the automated isolation of bacterial DNA from enrichment cultures of food samples using the KingFisher® Flex instrument.

Product No. KIT230184

Kit for 480 isolations

Store at 15 to 25 °C

For food testing purposes.

FOR *IN VITRO* USE ONLY

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1. Kit Components

All solutions, except for the foodproof® Magnetic Preparation Kit IV Magnetic Beads (bottle 5), are clear and should not be used when precipitates have formed. If precipitates have formed, warm the solutions in a 37 °C water bath until the precipitates have dissolved.

1.1 Kit Contents

Bottle / Tube	Label	Contents / Function
Bottle No. 1	foodproof Magnetic Preparation Kit IV Lysis Buffer	• 300 mL • For lysis of cells and extraction of DNA
Bottle No. 2	foodproof Magnetic Preparation Kit IV Wash Buffer I	• 330 mL, add 200 mL absolute isopropanol • For removing impurities
Bottle No. 3	foodproof Magnetic Preparation Kit IV Wash Buffer II	• 2 x 315 mL, add 2 x 210 mL absolute isopropanol • For removing impurities
Bottle No. 4	foodproof Magnetic Preparation Kit IV Elution Buffer	• 30 mL • For elution of DNA
Bottle No. 5	foodproof Magnetic Preparation Kit IV Magnetic Beads	• 2 x 8 mL • For binding of DNA
Tube No. 6	foodproof Magnetic Preparation Kit IV Lysozyme	• 10 x 11 mg; lyophilized • For digestion of bacterial cell wall. • Dissolve the lysozyme by addition of 1.1 mL of ddH_2O, mix thoroughly and store as described below!

1.2 Chemical Hazard

The foodproof Magnetic Preparation Kit IV Wash Buffer I (Bottle 2) contains irritating compounds that are harmful when brought in contact with skin, inhaled or swallowed. Always store and use these kit components away from food for humans and animals. Always wear gloves and follow standard safety precautions during handling.

1.3 Number of Preparations

480 isolations

1.4 Storage

All buffers and kit components of the foodproof Magnetic Preparation Kit IV should be stored at 15 to 25 °C (room temperature) and are stable through the expiration date printed on the label.

Lysozyme: Dissolved lysozyme should be stored in aliquots at –15 to –25 °C.

Wash Buffer I and II: Wash buffers charged with isopropanol should be stored at room temperature and must be sealed accordingly. If any precipitates are visible within the provided solutions, dissolve them by carefully warming.

2. Product Overview

2.1 Test Principle

The foodproof Magnetic Preparation Kit IV, in combination with the KingFisher® Flex instrument, provides fully automated purification of bacterial DNA from up to 400 µL from enrichment cultures of food samples (raw material and processed food). The DNA isolation process is based on 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 is suitable for direct use in PCR applications.

The KingFisher Flex instrument performs all steps of the DNA purification procedure automatically. The procedure requires only minimal interaction by the user, 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.

The KingFisher Flex instrument uses 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 direct liquid handling and increasing automation results in a fast, reliable and robust technique.

To achieve an efficient lysis and high DNA yields, the samples are first lysed with optimized lysis buffer and lysozyme. After lysis, the DNA binds to the magnetic beads, whereas contaminations, metabolites and enzyme inhibitors are efficiently removed during the following three wash steps. Finally, highly purified DNA is eluted in the elution buffer.

The purified, high-quality DNA is ready to use in downstream applications like PCR or can be stored at –20 °C for subsequent use.

2.2 Basic Steps

Step	Description
1	Sample is lysed by incubation with the lysis buffer and lysozyme
2	DNA is bound to magnetic beads
3	Bound DNA is washed to remove proteins and other cellular impurities
4	Purified DNA is recovered using the elution buffer

2.3 Sample Material

400 µL enrichment culture of food samples (raw material and processed food).

2.4 Quality Control

- Buffered peptone water spiked with approximately 5 x 10^4 CFU/mL of *C. sakazakii* is extracted and purified as described below.
- 5 µL of the eluate is analyzed using the foodproof *Enterobacteriaceae* plus *Cronobacter* Detection Kit (LC 480) (Product No. KIT230068-69).
- An additional DNA preparation and subsequent PCR setup of an unspiked broth sample is used as a negative quality control against contaminating DNA.

3. Procedures and Required Materials

3.1 Working Solutions and Additional Equipment

Before starting a run, bring all reagents to room temperature. Gently mix and redissolve any precipitates by warming up to 37 °C. Swirl gently to avoid foaming.

foodproof Magnetic Preparation Kit IV Lysis Buffer, Elution Buffer and Magnetic Beads are ready-to-use.

Add the required amount of ddH_2O to the reaction tube containing the foodproof Magnetic Preparation Kit IV Lysozyme and vortex for 5 s. Store unused and diluted foodproof Magnetic Preparation Kit IV Lysozyme at –20 °C.

Bottle	Content	Preparation of Working Solution	Storage and Stability
2	foodproof Magnetic Preparation Kit IV Wash Buffer I	Add 200 mL absolute isopropanol to the Wash Buffer I bottle. Mix thoroughly and always keep the bottle firmly closed. **Note:** Check the box on the label of the bottle after isopropanol has been added. Add the date for verifiability.	Store at room temperature. Stable until the expiration date printed on kit label.
3	foodproof Magnetic Preparation Kit IV Wash Buffer II	Add 210 mL absolute isopropanol to the Wash Buffer II bottle. Mix thoroughly and always keep the bottle firmly closed. **Note:** Check the box on the label of the bottle after isopropanol has been added. Add the date for verifiability.	Store at room temperature. Stable until the expiration date printed on kit label.
6	foodproof Magnetic Preparation Kit IV Lysozyme	Dissolve the lysozyme with 1.1 mL of double-distilled water (ddH_2O) per reaction tube needed.	Store at –20 °C, stable for 12 months.

Additional Equipment and Reagents required:

- KingFisher Flex instrument
- Pipette and pipette tips
- Disposable gloves
- ddH_2O
- Vortexer
- Absolute isopropanol (96 – 98%)
- Deep-well plates
- Elution plates
- Tip Comb 96 DWH

All necessary plastic consumables are available through Hygiena Diagnostics.

3.2 Protocol

Caution
Always wear gloves during the procedure and follow safety precautions to minimize contact when handling. Follow generally applicable safety precautions regulating the work with biohazard materials. Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

The following protocol describes the automated DNA isolation from 400 µL sample material with the KingFisher Flex instrument:

1. Switch on the KingFisher Flex instrument.

   Note: Before starting the purification process with the KingFisher Flex instrument, carefully read the user manual!

   Resuspend and vortex the Magnetic Beads thoroughly before use!

2. **Tip Plate:** Place the Tip Comb 96 DWH on a Tip Plate. (Use one Elution Plate as the Tip Plate.)
3. Prefill the Binding Plate, the Washing Plates and the Elution Plate as described below:
   a. **Binding Plate**: Add 500 µL **Lysis Buffer,** 20 µL **Lysozyme** and 30 µL **Magnetic Beads**
   b. **Washing Plate I:** Add 1,000 µL **Wash Buffer I**
   c. **Washing Plate II:** Add 1,000 µL **Wash Buffer II**
   d. **Washing Plate III:** Add 1,000 µL **Wash Buffer II**
   e. **Elution Plate**: Add 50 µL **Elution Buffer**
4. Transfer 400 µL of the sample into the Binding Plate.
5. Choose assay file ‘foodproof_MPK_IV’ on the instrument and press ‘START’.
6. Follow instructions on the instruments display and load the prefilled buffer plates in the right position. Confirm with ‘START’ after each loading step; the instrument will then provide the next available loading position automatically.
7. When all plates are loaded, press ‘START’ again to initialize the program.

The following purification steps will run automatically on the KingFisher Flex instrument:

1. **Cell Lysis:** Cells are lysed for 20 min by continuously mixing.
2. **DNA Binding:** Sample is mixed automatically for 5 min. Magnetic beads are separated and transferred to Washing Plate I.
3. **First Wash:** Sample is mixed automatically for 30 s. Magnetic beads are separated and transferred to Washing Plate II.
4. **Second Wash:** Sample is mixed automatically for 30 s. Magnetic beads are separated and transferred to Washing Plate III.
5. **Third Wash:** Sample is mixed automatically for 30 s. Magnetic beads are separated.

6. **Drying:** The magnetic beads are dried outside of Washing Plate III for 7 min and transferred to the Elution Plate.
7. **DNA Elution:** Magnetic particles are incubated in Elution Buffer for 10 min at 90 °C by continuously mixing. Magnetic beads are automatically separated, removed and transferred to Washing Plate III (disposal).

Note*:*

- *At the end of the extraction protocol, the Elution Plate contains the extracted DNA.*
- *If the extracted DNA contains carryover of magnetic particles, transfer the DNA into a 1.5 mL reaction tube and centrifuge at maximum speed for 1 min. Transfer the clear supernatant (contains the DNA) into a new tube.*
- *Storage of Samples:*

If you want to…	Then
Continue	Use the eluted DNA directly
Stop	The eluted DNA can be stored at 4 to 8 °C for one week or at –20 °C for long-term storage.

3.3 Self-programming of the KingFisher Flex instrument

3.3.1 Protocol information

Protocol name: foodproof_MPK_IV
Kit name: foodproof MPK IV
Description: KingFisher Flex protocol for isolation of bacterial DNA from enrichment cultures from raw material and food products.

3.3.2 Plate Layouts

Binding Plate		**Microtiter 96 Deep Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
Sample	400	-	Sample
Lysis Buffer	500	-	Reagent
Lysozyme	20	-	Reagent
Magnetic Beads	30	-	Reagent
Washing Plate I		**Microtiter 96 Deep Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
Wash Buffer I	1000	-	Reagent
Washing Plate II		**Microtiter 96 Deep Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
Wash Buffer II	1000	-	Reagent
Washing Plate III		**Microtiter 96 Deep Well Plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	1000	-	Reagent
Elution Plate		**Microtiter 96 Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
Elution Buffer	50	-	Reagent
Tip Plate		**Microtiter 96 Well Plate**	
Name	**Well volume [µL]**	**Total reagent volume [µL]**	**Type**
-	-	-	-

3.3.3 File Steps

Tip 1		**Tip Comb 96 DWH**	
	Pick-Up	**Tip Plate**	
	Lysis	**Binding Plate**	
	Beginning of step:	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:20:00, Slow
		Heating temperature [°C]	55
		Preheat	Yes
	End of step:	Postmix	No
		Collect beads	No
	Binding	**Binding Plate**	
	Beginning of step:	Precollect	No
		Release beads	No
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step:	Postmix	No
		Collect count	2
		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	00:00:30, Medium
		Heating during mixing	No
	End of step:	Postmix	No
		Collect count	2
		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	00:00:30, Medium
		Heating during mixing	No
	End of step:	Postmix	No
		Collect count	2
		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	00:00:30, Medium
		Heating during mixing	No
	End of step:	Postmix	No
		Collect count	1
		Collect time [s]	3
	**Dry1**	**Washing Plate III**	
		Dry time	00:07:00
		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	00:05:30, Slow
		Heating temperature [°C]	90
		Preheat	No
	End of step:	Postmix time	00:04:00, Fast
		Collect count	1
		Collect time [s]	3
	**ReleaseBeads1**	**Washing Plate III**	
		Release time, speed	00:00:10, Bottom mix
	**Leave**	**Tip Plate**	

4. Typical Results

Purified DNA is free of other cellular components and DNA polymerase inhibitors.

5. Troubleshooting

Problem	Possible Cause	Recommendation
Low DNA yield or purity	Kit stored under non-optimal conditions.	• Store diluted lysozyme 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
	Isopropanol not added to Wash Buffer I or Wash Buffer II.	• Add absolute isopropanol to Wash Buffer I and Wash Buffer II before using • After adding isopropanol, mix Wash Buffer I and Wash Buffer II well, and store at room temperature • Always mark Wash Buffer I and Wash Buffer II bottles to indicate the addition of isopropanol
	Low amount of Magnetic Beads	• Mix the Magnetic Beads thoroughly before pipetting into the Binding Plate
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 A_{260}:A_{280} ratio from UV measurement, eluted DNA is brown colored	Some of the magnetic particles are left in the elution	• Centrifuge at full speed for 1 min and transfer supernatant, which contains the DNA, to a new tube

6. Appendix

6.1 KingFisher Flex Software 3.2

The KingFisher Flex Software 3.2 creates assay files for the KingFisher Flex instrument. The respective assay file can either be transferred onto the KingFisher Flex workstation or be started directly from within the BindIt software.

Note: Assay files run directly with BindIt will not be stored in the workstation memory!

7. Warranty and Disclaimer of Liability

Limited Warranty and Disclaimer of Liability. Hygiena Diagnostics 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 conditions are complied with:

(1) The product is used according to the guidelines and instructions set forth in the product literature;

(2) Hygiena Diagnostics does not warrant its product against any defects when: defect is a result of material or workmanship not provided by Hygiena Diagnostics; defect is caused by misuse or use contrary to the instructions supplied; the product is contaminated by improper storage or handling;

(3) All warranties of merchantability and suitability 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 the conditions described here;

(4) Hygiena Diagnostics does not assume 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 expressed, unless deviating terms are expressed in writing by an officer of Hygiena Diagnostics;

(5) Hygiena Diagnostics 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 reserves the right to replace or allow credit for any modules returned under this warranty.

8. Supplementary Information

8.1 Ordering Information

Hygiena Diagnostics offers a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.hygiena.com.

8.2 Trademarks

foodproof® is a registered trademark of Hygiena Diagnostics GmbH. Hygiena® is a registered trademark of Hygiena.

KingFisher® is a registered trademark of Thermo Fisher Scientific. Other brand or product names are trademarks of their respective holders.

8.3 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, please contact our Technical Support staff (www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. Contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

8.4 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 15L

9. Change Index

Version 1, August 2014
First version of the package insert.

Revision A, January 2024
Rebranding and new layout.
S 400 15L 20 -> INS-KIT-230184-REVA

Revision B, November 2024
Updated Magnetic Bead storage conditions.

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