For testing of food and environmental samples

foodproof® Magnetic Preparation Kit I

Revision A, July 2023

Application manual for the automated isolation of Gram-negative bacterial DNA from enrichment cultures of food samples using the **food**proof® RoboPrep® Series or the KingFisher™ Flex instrument

Product No. KIT230180

Kit for 480 isolations

Store the kit at 15 to 25 °C

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1. Kit Components

All solutions except foodproof Magnetic Preparation Kit I Lysis Buffer (Bottle 1) and foodproof Magnetic Preparation Kit I Binding Buffer (Bottle 2) 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.

Chemical Hazard

The foodproof Magnetic Preparation Kit I Lysis Buffer (Bottle 1), foodproof Magnetic Preparation Kit I Binding Buffer (Bottle 2) and foodproof Magnetic Preparation Kit I Wash Buffer I (Bottle 3) contain irritating compounds that are harmful when brought into contact with skin or 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.

Number of Preparations

480 isolations

Storage

Store the kit at 15 to 25 °C through the expiration date printed on the label.

Note: Inappropriate storage at 2 to 8 °C (refrigerator) or -25 to -15 °C (freezer) will adversely affect nucleic acid purification when precipitates form in the solutions.

Kit Contents

Bottle	Label	Contents / Function
1	foodproof Magnetic Preparation Kit I Lysis Buffer	• 205 mL • For lysis of cells and extraction of DNA
2	foodproof Magnetic Preparation Kit I Binding Buffer	• 120 mL, add 80 mL absolute isopropanol. • For binding of DNA to the magnetic beads
3	foodproof Magnetic Preparation Kit I Wash Buffer I	• 254 mL, add 154 mL absolute isopropanol • For removing impurities
4	foodproof Magnetic Preparation Kit I Wash Buffer II	• 246 mL, add 164 mL absolute isopropanol • For removing impurities
5	foodproof Magnetic Preparation Kit I Wash Buffer III	• 410 mL • For removing impurities
6	foodproof Magnetic Preparation Kit I Elution Buffer	• 192 mL • For elution of DNA

2. How to Use this Product
2.1 Product Overview

Test Principle

The foodproof Magnetic Preparation Kit I, in combination with the foodproof RoboPrep^{+} workstation or the foodproof RoboPrep Fusion system, provides fully automated purification of total genomic bacterial DNA from enrichment cultures of food samples. The kit provides high-quality DNA, which is suitable for direct use in PCR applications. The foodproof RoboPrep^{+} Series workstation or the foodproof RoboPrep Fusion system performs all steps of the sample preparation procedure and can also perform the PCR setup procedure.

Additionally, the foodproof Magnetic Preparation Kit I,, in combination with the foodproof RoboPrep 32 or the KingFisher™ Flex instrument, provides semi-automated purification of total genomic bacterial DNA from enrichment cultures of food samples.

The cells are lysed during a short incubation with the provided foodproof Magnetic Preparation Kit I Lysis Buffer. After addition of the foodproof Magnetic Preparation Kit I Binding Buffer, the DNA selectively binds to the magnetic beads. Bound DNA is purified in three washing steps to remove potential PCR inhibitors. Then, a low-salt elution buffer releases the DNA from the magnetic beads. This simple method eliminates the need for organic-solvent extractions and DNA precipitation, thus providing rapid, simultaneous purification of many samples.

Basic Steps

Step	Description
1	Cells are lysed by incubation with foodproof Magnetic Preparation Kit I Lysis Buffer
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 foodproof Magnetic Preparation Kit I Elution Buffer

Application

The foodproof Magnetic Preparation Kit I is optimized for isolation of bacterial DNA from enrichment cultures of various food samples (raw material and processed food). The kit is optimized for gram-negative bacteria. The quality of the DNA obtained with the kit is highly suitable for applications using any PCR System.

Note: For protein-rich food samples like egg, ham, beef, pork, chicken, minced meat, salmon and cheese, the addition of Reagent P (Product No. KIT 2300 07) to the foodproof Magnetic Preparation Kit I Lysis Buffer is necessary. The preparation of the working solution for this application is described in detail in the manual of the Reagent P.

Sample Material

0.2 mL enrichment culture of food samples (raw material and processed food).

Quality Control

- 5 x 10^4 CFU/ml *Salmonella enterica* subsp. *enterica* serovar Senftenberg is extracted and purified as described below.

- 5 µL of the eluate is analyzed using the foodproof *Salmonella* Detection Kit (Product No. KIT 2300 49). As expected, the resulting amplification signal is obtained.
- 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 Before You Begin

Preparation of Kit Working Solutions

In addition to the ready-to-use solutions supplied with the kit, you will need the following working solutions; preparation of working solutions is required:

Bottle	Content	Preparation of working solution	Storage and stability
2	Binding Buffer	Add 80 mL absolute isopropanol to foodproof Magnetic Preparation Kit I Binding Buffer. **Note:** Check the box on the label of the bottle after isopropanol has been added. Add the date for verifiability.	Store at 15 to 25 °C. Stable until the expiry date printed on kit label.
3	Wash Buffer I	Add 154 mL absolute isopropanol to foodproof Magnetic Preparation Kit I Wash Buffer I. **Note:** Check the box on the label of the bottle after isopropanol has been added. Add the date for verifiability.	Store at 15 to 25 °C. Stable until the expiry date printed on kit label.
4	Wash Buffer II	Add 164 mL absolute isopropanol to foodproof Magnetic Preparation Kit I Wash Buffer II. **Note:** Check the box on the bottle label after isopropanol has been added. Add the date for verifiability.	Store at 15 to 25 °C. Stable until the expiry date printed on kit label.

3.2 Protocol for the foodproof RoboPrep⁺ workstation

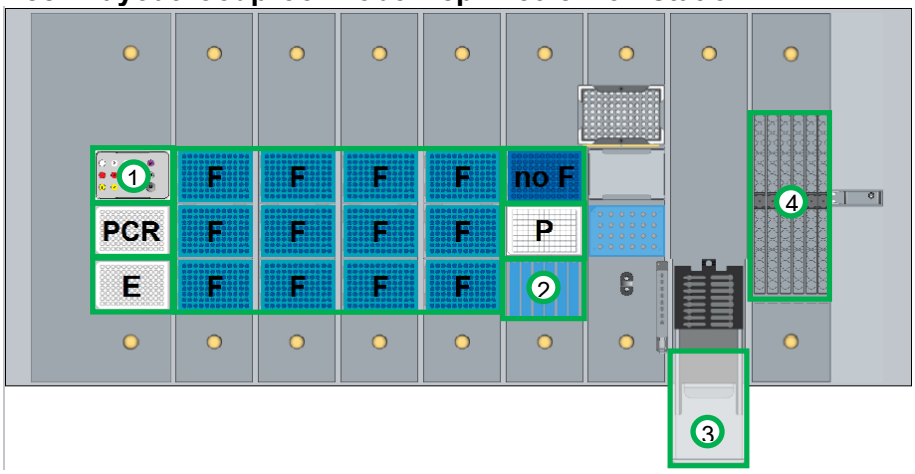
Additional Equipment and Reagents required

- foodproof RoboPrep⁺ workstation
- RAININ disposable filter tips, 250 µL
- RAININ disposable tips, 1,000 µL
- RAININ disposable filter tips, 1000 µL
- Eppendorf troughs (reagent holders)
- Process deep well plates
- Elution microplates
- PCR Cooler for 96-well plate
- 5 mL tubes (for master mix preparation)
- Disposable waste bags

- 12 mL sample tubes
- PCR reagents
- PCR plates
- Isopropanol, absolute
- Reagent P (Product No. KIT 2300 07), recommended for protein-rich food matrices

Placement of Reagents and Equipment

Deck-Layout foodproof RoboPrep+ 150-8 workstation:



Deck-Layout foodproof RoboPrep+ 100-8 workstation:

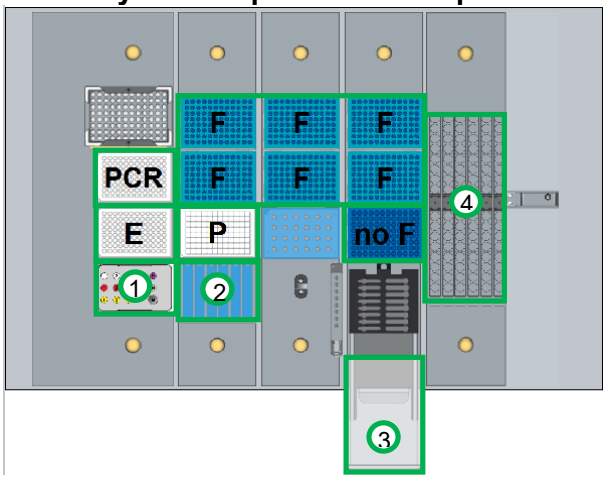


Figure 1. Positioning of reagents and equipment on the deck of the foodproof RoboPrep+ workstations.

1: PCR setup rack

2: Eppendorf Troughs, 6 reagent containers

3: Disposable waste bag

4: Rack for 12 mL sample tubes, up to 96 tubes

E: Elution micro plate

F: RAININ disposable filter tips, 1,000 µL

no F: RAININ disposable tips, 1,000 µL

P: Process deep well plate

PCR: PCR plate

Placement Procedure

1. **Place the PCR plate, the elution micro plate and the process deep well plate at the appropriate starting positions (PCR, E and P).**
2. **Place the disposable waste bag at the appropriate position (3).**
3. **Load the metal racks with tips (1000 µL) (F, no F)**

Note: For placement of tips in the workstation, pay attention to the motion sequence of the workstation's pipetting arm during the tip pick-up process. It starts with the top right tip rack and ends with the bottom left one (see Fig. 2). Within each tip rack, single tips are picked up the opposite way (see Fig. 3).

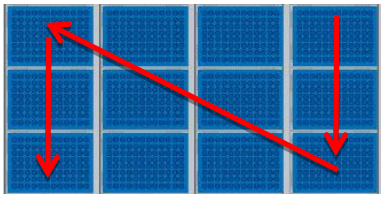


Figure 2. Directions for placing tips on the deck

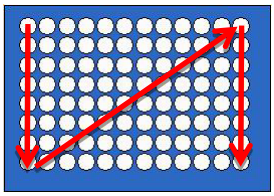


Figure 3. Tip pickup direction within tip racks

4. **Load the sample tubes into the rack (4)**

Note: The sample tubes will be used starting with the top left tube and ending with the bottom right tube (see Fig. 4).

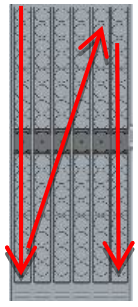


Figure 4. Directions for positioning sample tubes

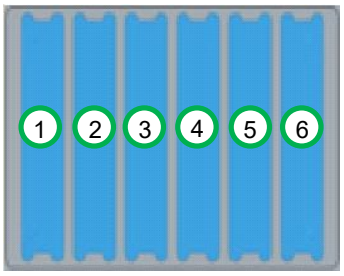
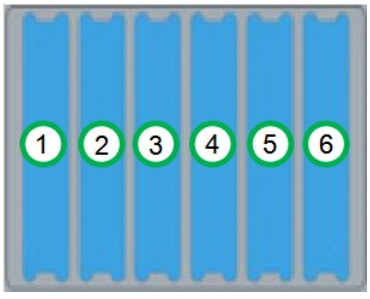


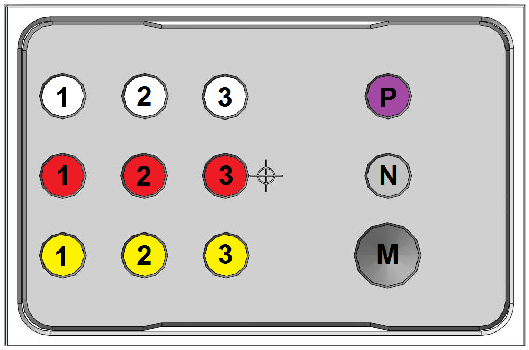
Figure 5. Reagent containers

5. **Load the reagent containers with the following kit components (3, Fig. 5)**



1: Lysis Buffer
2: Binding Buffer
3: Wash Buffer I
4: Wash Buffer II
5: Wash Buffer III
6: Elution Buffer

6. **Load the PCR setup rack with the following PCR kit components (1, Fig. 6)**



White: Tubes with Internal Control.
Red: Tubes with Enzyme Solution
Yellow: Tubes with Master Mix
P: Control Template
N: Negative control, PCR-grade H_2O
M: 5 mL tube for PCR Mix

Figure 6. PCR setup rack.

Caution
Always wear gloves during the procedure and follow safety precautions to minimize contact when handling. Follow all universal safety precautions governing work with biohazardous materials (e.g., wear lab coats at all times). Also, 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 and additional PCR setup from 0.2 mL enrichment culture with the foodproof RoboPrep⁺ workstation.

1. **Place 5 – 10 mL of food enrichment culture into 12 mL sample tubes.**
2. **Switch on the foodproof RoboPrep⁺ workstation and monitor and allow the system to boot up.**
   The power switch is on the back side of the instrument.
3. **Double-click the lirix3 shortcut icon on the desktop.**
4. **The log in screen will be displayed (Fig. 7).**

Figure 7. Lirix3 log in dialog window

5. **Enter user name and password.**
   Note that a password was set for all users in the access control manager.
6. **Select OK.**
7. **The screen "Application Setup – Methods" will be displayed (Fig. 8).**

Note that only those categories to which the logged on user has access rights will be displayed and active.

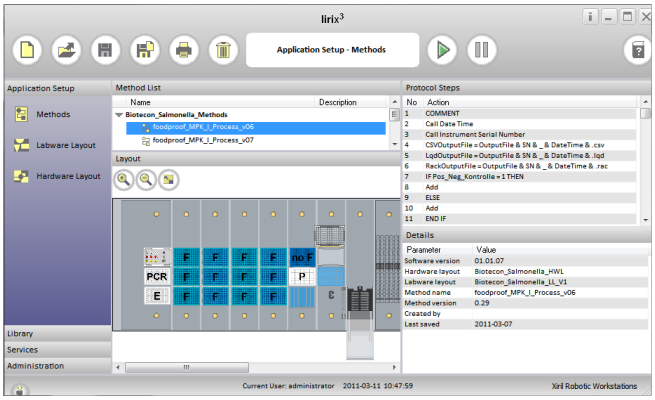


Figure 8. Application Setup - Methods window

8. **Select method "foodproof_MPK_I_Process_vxx"**
   The available methods are grouped under headings.

   To open a group, select the heading required. To select a method, left-click on the method name.

   Choose the method "**foodproof_MPK_I_Process_v06"** for food samples with low protein content -or-
   Choose the method "**foodproof_MPK_I_Process_v07"** for protein-rich food samples.

9. **Start the method by clicking on the run button**  **.**

10. **Select input variables from the "Input Variables" dialog window (Fig. 9).**

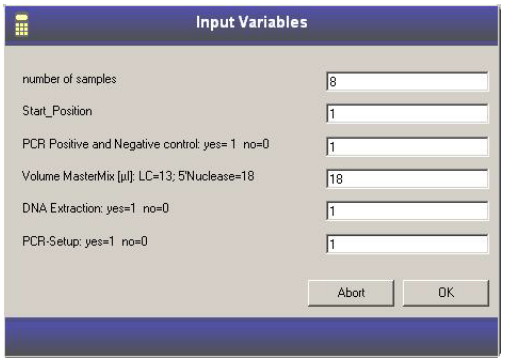


Figure 9. Input Variables - dialog window

11. **Choose the number of samples: 1 – 96.**
12. **Choose the start position.**
13. **For PCR setup, choose whether a positive and a negative control should be included.** If yes, PCR master mix will be prepared for one positive and one negative control each in addition to the number of samples. If not, PCR master mix will be prepared for the processed number of samples only.
14. **Choose the appropriate volume for the Master Mix according to the used PCR Kit:** If you are using a foodproof Detection Kit based on 5' Nuclease technology, choose 18. If you are using a foodproof Detection Kit based on Hybridization Probes technology, choose 13.
15. **Choose whether you want to conduct DNA extraction.**
16. **Choose whether you want to conduct a PCR setup. Click OK to continue.**
17. **In the "Start Run" dialog window (Fig. 10), you can choose to start the run with a new tip rack.** If you do so, tips will be taken starting from the first position of the first tip rack defined in the labware layout. If not, tips will be taken starting from the next available tip after the last used tip position. Note that switching from one method to another with a different process layout resets the used tip position back to the first position in the first rack.

Figure 10. "Start Run" dialog window

18. **The instrument is initialized and the run starts.**
19. **Place the sample tubes into the sample tube racks.**

20. Request for all necessary equipment and reagents.

The following dialog windows will be displayed and the software will now guide you through the remaining steps required to set up the RoboPrep^{+} workstation for the "foodproof Magnetic Preparation Kit I Process".

21. Check for the necessary volume of Lysis Buffer (Fig. 11).

Fill the necessary volume of Lysis Buffer into the first reagent reservoir and confirm with "continue".

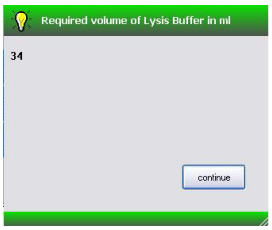


Figure 11. Requested volume of Lysis Buffer - dialog window

22. Check for the necessary volume of Binding Buffer (Fig. 12).

Fill the necessary volume of Binding Buffer into the second reagent reservoir and confirm with "continue".

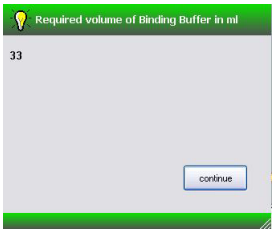


Figure 12. Requested volume of Binding Buffer - dialog window

23. Check for the necessary volume of Wash Buffer I (Fig. 13).

Fill the necessary volume of Wash Buffer I into the third reagent reservoir and confirm with "continue".

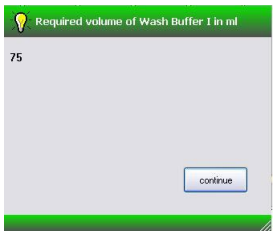


Figure 13. Requested volume of Wash Buffer I - dialog window

24. Check for the necessary volume of Wash Buffer II (Fig. 14).

Fill the necessary volume of Wash Buffer II into the fourth reagent reservoir and confirm with "continue".

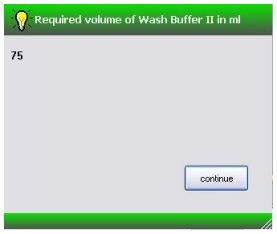


Figure 14. Requested volume of Wash Buffer II - dialog window

25. Check for the necessary volume of Wash Buffer III (Fig. 15).

Fill the necessary volume of Wash Buffer III into the fifth reagent reservoir and confirm with "continue".

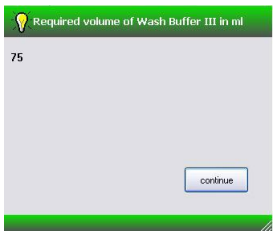


Figure 15. Requested volume of Wash Buffer III- dialog window

26. Check for the necessary volume of Elution Buffer (Fig. 16).

Fill the necessary volume of Elution Buffer into the sixth reagent reservoir and confirm with "continue".

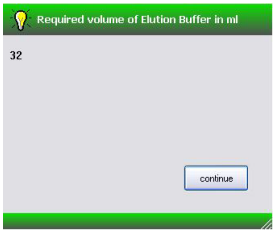


Figure 16. Requested volume of Elution Buffer - dialog window

27. Check for the necessary plates (Fig. 17).

Place the elution plate, the PCR plate and the process plate at the start positions and confirm with "continue".

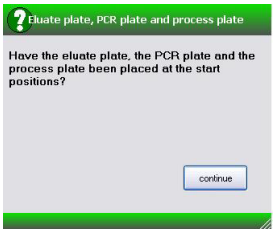


Figure 17. Requested plates - dialog window

28. Check for the necessary number of racks with filter tips (Fig. 18).

Place the necessary number of racks with filter tips on the deck beginning with the first tip rack defined in the labware layout and confirm with "continue".

Figure 18. Required racks with filter tips- dialog window

29. Check for the necessary number of tips without filters (Fig. 19).

Place the necessary number tips without filters into the defined rack and confirm with "continue".

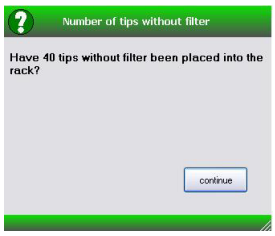


Figure 19. Number of tips without filter- dialog window

30. Check for the PCR reagents (Fig. 20).

Place the necessary PCR reagents into the PCR setup rack and confirm with "continue".

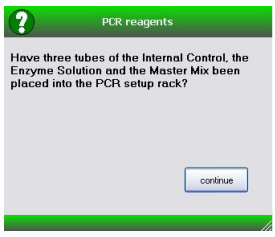


Figure 20. PCR reagents- dialog window

31. Check for the 5 mL tube for the Master Mix preparation (Fig. 21).

Place the 5 mL tube into the PCR setup rack and confirm with "continue".

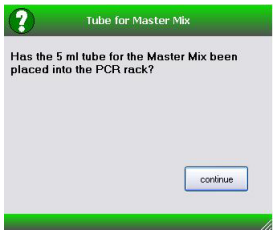


Figure 21. Tube for Master Mix- dialog window

32. **All next steps are fully automated, and a software message on the screen will indicate when the protocol is finished (Fig. 22).**

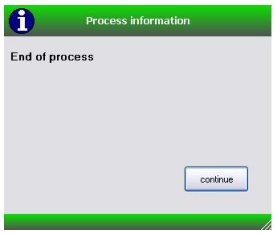


Figure 22. Process information- dialog window

Additional Information:

- The protocol steps window shows the list of programmed actions and the running action is highlighted.
- Pause and continue a run: To pause or abort a process, click on the pause icon on the screen or press the pause key. The RoboPrep+ workstation completes the *current* action before pausing. A dialog then prompts the user to abort or continue the method.

Storage of Samples

If you want to…	Then
Continue	Use the eluted DNA directly
Stop	Store the DNA at -25 to -15 °C for later analysis

3.3 Protocol for the foodproof RoboPrep Fusion system

The foodproof RoboPrep Fusion is the combination of the JANUS® G3 Integrator instrument from PerkinElmer® and the KingFisher™ Flex instrument from Thermo Fisher Scientific. The foodproof RoboPrep Fusion is controlled by the WinPREP® software of the PE JANUS G3 instrument.

Additional Equipment and Reagents required

- 175 µL Conductive Filter Tips
- 900 µL Conductive Filter Tips
- 1 Well Liquid Handling Reservoir
- 4 Well Liquid Handling Reservoir
- Universal Lid for all 96-well Plates
- 10 mL Sample Tubes, sterile
- KingFisher 96 tip comb for DWH
- KingFisher 96 Deepwell Plate, sterile
- KingFisher 96 Plate, 200 µL
- Sealing foil
- Disposable gloves
- ddH_2O
- Vortex
- Absolute isopropanol (96-98 %)

All necessary plastic consumables are available through Hygiena Diagnostics GmbH.

Running the protocol on the foodproof RoboPrep Fusion

Caution

Always wear gloves during the procedure and follow safety precautions to minimize contact when handling. Follow all universal safety precautions governing work with biohazardous materials (e.g., wear lab coats at all times). Also, properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

JANUS Application Assistant guides you through the entire process of selecting and running the protocol.

To open JANUS Application Assistant click on this icon located on your Windows desktop:

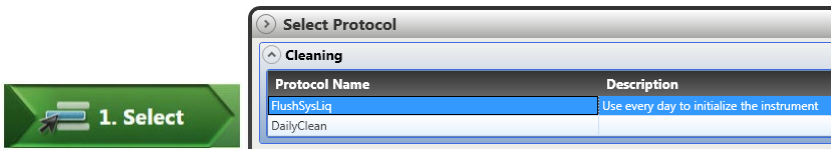
A. Daily Preventative Maintenance

Flushing the Varispan™ system of the foodproof RoboPrep Fusion with degassed distilled water helps to keep the system free of air bubbles, crystals, precipitates, and biological growth that can accumulate within the tubing, valves, and syringes. If allowed to accumulate in the liquid path, these items decrease the accuracy and precision of the instrument. To prevent this problem, **flush the system at the start of each working day**.

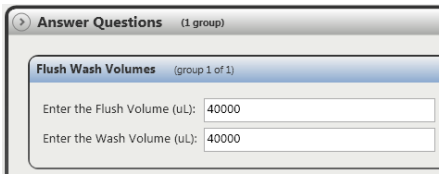
To flush the system:

1. Fill the system liquid container with degassed, distilled water.

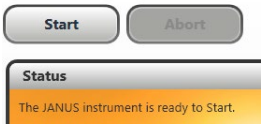
2. Clicking on the Select button and choosing the protocol "FlushSysLiq" under the Select Protocol section "Cleaning" of the window to start the flush process.



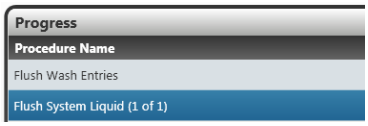
3. Set both the Flush and Wash volumes to 40,000 µL, when prompted.



4. Click on the Run button and the Start button to start the process.

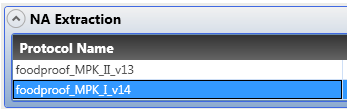


5. While the protocol is running, the relative status of the protocol is constantly updated on the screen by the Progress panel.

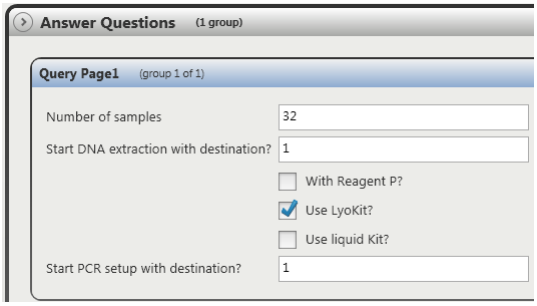


B. Executing the foodproof MPK I protocol

1. Click on the Select button and choose the protocol "foodproof_MPK_I_vxx" under the Select Protocol section "NA Extraction" of the window.



2. Respond to the questions that are associated with the selected protocol. The questions are listed under the Answer Questions section of the window. You must respond to these questions before you move on to the next step. The answers that you provide help to govern the successful execution of the protocol:

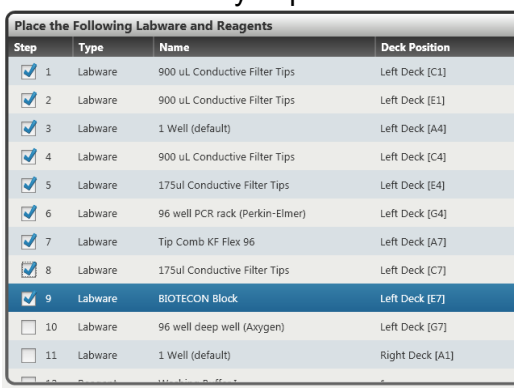


3. Proceed to Step 2: Click on the Next Step button or the Gather button:

4. Inspect the checklist (under the section 'Gather the Following Labware and Reagents') and collect the labware that you will need to run the protocol. Click on each labware item that you collect. All labware that is listed is required. For your convenience, the location of a lab item or reagent may be listed. This should shorten the time it takes to find the item.

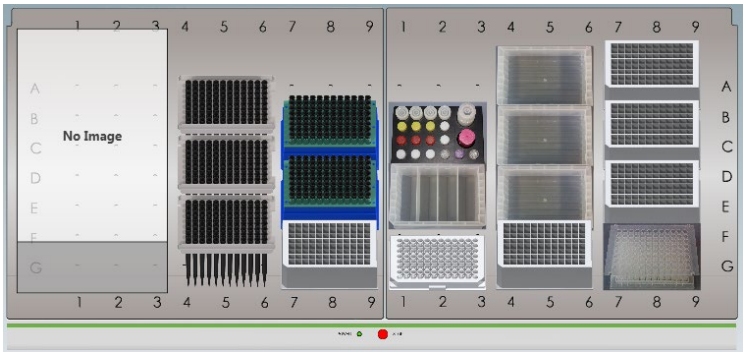
5. Proceed to Step 3. Click on the Next Step button or the Place button:

6. Next, you need to populate the instrument deck with the labware items that you collected in Step 4. The collected labware and reagents are listed under the 'Place the Following Labware and Reagents' section of the window. The Deck Position of each item is also listed. Click on each labware item that you place on the deck:



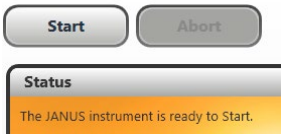
7. When you select an item in the top left section of the window 'Place the Following Labware and Reagents', the item's placement instructions are displayed under the Instructions section of the window:

The bottom portion of the window offers a panoramic view of the entire populated deck:

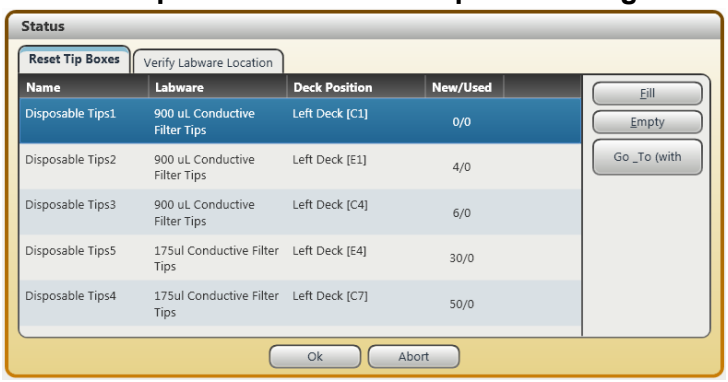


8. Pipet 5 – 10 mL of the food enrichment culture into a 12 mL sample tube and place it into the sample tube rack.

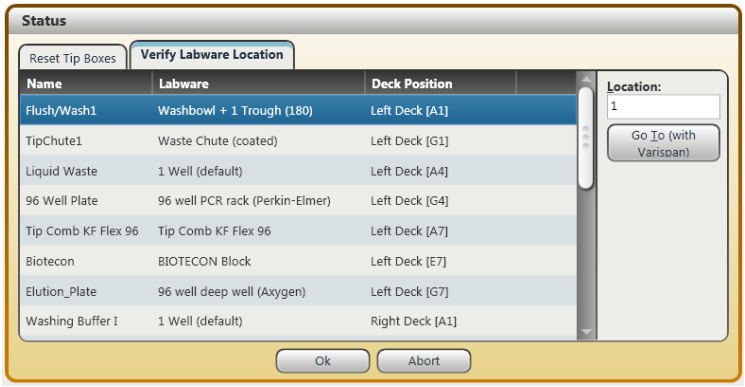
9. Run the protocol and monitor its progress as the protocol executes.



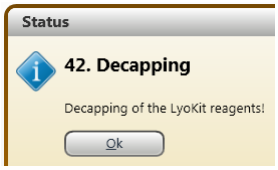
10. Reset tip boxes or start the tips remaining from a previous run.



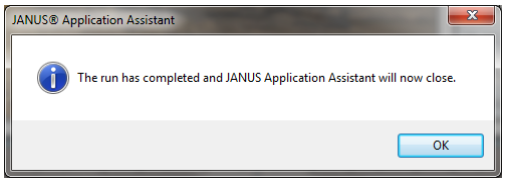
11. Additionally, the list of labware can be verified.



12. Start PCR Setup for LyoKits after decapping the PCR tubes.



13. The following window indicates that the protocol is finished.



14. Clean up the instrument after the successful execution of the protocol.

Storage of Samples

If you want to…	Then
Continue	Use the eluted DNA directly
Stop	Store the DNA at -25 to -15°C for later analysis

The following protocol describes the automated DNA isolation and additional PCR setup from 0.2 mL enrichment culture with the foodproof RoboPrep Fusion:

1. Prefilling of the KingFisher Flex plates by the PerkinElmer® JANUS G3 instrument:

   Elution Plate: 300 µL Elution Buffer added

   Washing Plate I: 750 µL Wash Buffer I added

   Washing Plate II: 750 µL Wash Buffer II added

   Washing Plate III: 750 µL Wash Buffer III added

   Lysis Plate: 320 µL Lysis buffer (+ 25 µL Reagent P, optional)
2. Transfer of 200 µL sample into the Lysis Plate.
3. Transport of the KingFisher Flex plates to the KingFisher Flex instrument
4. Execution of the foodproof MPK I program on the KingFisher Flex instrument
5. After an elevated lysis step of 10 min, a pause step occurs. The Lysis Plate is automatically transported to the PerkinElmer® JANUS G3 instrument.
6. Lysis Plate: 315 µL Binding Buffer added
7. Transport of the Lysis plate to and continuation of the program on the KingFisher Flex instrument
8. After finishing the extraction protocol, the Elution Plate containing the extracted DNA and the other plates are transported back to the PerkinElmer JANUS instrument.
9. Execution of the PCR setup in the PE JANUS instrument

The program of the KingFisher Flex system consists of the following steps:

- Lysis of cells: Cell lysis for 10 min by continuously mixing.
- Binding of the DNA: Automatically sample mixing for 5 min. Magnetic beads separation. Transfer of the magnetic particles to Washing Plate I.
- First Washing: Automatically sample mixing for 1 min. Magnetic beads separation. Transfer of the magnetic particles to Washing Plate II.
- Second Washing: Automatically sample mixing for 1 min. Magnetic beads separation. Transfer of the magnetic particles to Washing Plate III.
- Third Washing: Automatically sample mixing for 20 s. Magnetic beads separation. Transfer of the magnetic beads to the Elution Plate.
- Elution of the DNA: Incubation of magnetic particles in the Elution Buffer for 10 min 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 KingFisher Flex instrument

Protocol information

Protocol name: foodproof_MPK_I_v02_Fusion
Kit name: foodproof MPK I
Description: KingFisher Flex protocol for isolation of genomic DNA from Gram-negative bacteria from enrichment cultures from raw material and food products.

Plate layouts

Tip Plate		KingFisher 96 KF plate	
Name	Well volume [µL]	Total reagent volume [µL]	Type
-	-	-	-
Lysis Plate		**Microtiter DW 96 plate**	
Name	Well volume [µL]	Total reagent volume [µL]	Type
Sample	200	-	Sample
Lysis Buffer	320	-	Reagent
Washing Plate 1		**Microtiter 96 DW plate**	
Name	Well volume [µL]	Total reagent volume [µL]	Type
Wash Buffer I	750	-	Reagent
Washing Plate 2		**Microtiter 96 DW plate**	
Name	Well volume [µL]	Total reagent volume [µL]	Type
Wash Buffer II	750	-	Reagent
Washing Plate 3		**Microtiter 96 DW plate**	
Name	Well volume [µLl]	Total reagent volume [µL]	Type
Wash Buffer III	750	-	Reagent
Elution Plate		**Microtiter 96 DW plate**	
Name	Well volume [µL]	Total reagent volume [µL]	Type
Elution Buffer	300	-	Reagent

Dispensed reagents

Lysis Plate		Microtiter DW 96 plate	
Name	Step	Well volume [µL]	Total reagent volume
Binding Buffer	Adjust Binding	315	-

File Steps

	Tip 1	**Tip Comb 96 DWH**	
	Pick-Up	**Tip Plate**	
	Lysis Step	**Lysis Plate**	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	95
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Adjust Binding	**Lysis Plate**	
		Message	Add Binding Buffer
		Dispensing volume [µl]	315
	Reagent(s)	Name	Binding Buffer
		Volume [µl]	315
	Pause 1	**Lysis Plate**	
		Message	Wait for Flex
	Binding	**Lysis 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	4
		Collect time [s]	3
	Washing_1	**Washing Plate 1**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast

	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	**Washing_2**	**Washing Plate 2**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	**Washing_3**	**Washing Plate 3**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:00:20, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	5

	Elution	**Elution Plate**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	90
		Preheat	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	15
	Bead Removal	**Washing Plate 3**	
		Release time, speed	00:00:30, Fast
	Pause 2	**Lysis Plate**	
		Message	Wait for Flex
	Leave	**Tip Plate**	

3.4 Protocol for the semi-automated DNA extraction with the KingFisher Flex

Additional Equipment and Reagents required

- KingFisher Flex instrument
- Pipette and pipette tips
- Disposable gloves
- ddH_2O
- Vortex
- Absolute isopropanol (96-98 %)
- Microtiter 96 DW plate, 2.0 mL
- KingFisher 96 KF plate, 200 µL
- Tip Comb 96 DWH
- Adhesive Seal

All necessary plastic consumables are available through Hygiena Diagnostics GmbH.

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 semi-automated DNA isolation from 200 µL sample material with the KingFisher Flex instrument:

1. Switch on the KingFisher Flex instrument.

Note: Please read the user manual carefully before starting the purification process with the KingFisher Flex instrument! **Resuspend the Lysis Buffer and the magnetic beads in the Binding Buffer thoroughly directly before use!**

2. **Tip Plate:** Place the Tip Comb 96 DWH on a Tip Plate (Use one provided Elution Plate (200 µl) as Tip Plate.).
3. Prefill the Lysis Plate, the Washing Plates and the Elution Plate as described below:
4. **Lysis Plate**: Add **320 µL Lysis Buffer** and **25 µL Reagent P** (if necessary)
5. **Washing Plate I:** Add **750 µL Wash Buffer I**
6. **Washing Plate II:** Add **750 µL Wash Buffer II**
7. **Washing Plate III:** Add **750 µL Wash Buffer III**
8. **Elution Plate**: Add **300 µL Elution Buffer**
9. Transfer **200 µL** of the **sample** into the **Lysis Plate**.
10. Choose assay file **'foodproof_MPK_I_vxx'** on instrument and press "START".
11. 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 then will provide the next free loading position automatically.
12. When all plates are loaded, press "START" again to initialize the program. The program starts with the lysis of the sample.
13. After an elevated lysis step of 10 minutes a pause step occurs. The **Lysis Plate** is automatically moved to the loading position of the instrument. Take out the plate and add **315 µL Binding Buffer**. Reinsert the plate into the loading position (pay attention to the correct plate orientation; a disregard will result in a failed run) and press the "START" button to continue with the run. From this point on, the instrument will continue with the purification process without any further user interaction.

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

- **Lysis of cells:** Cell lysis for 10 min by continuously mixing.
- **Binding of the DNA:** Automatically sample mixing for 5 min. Magnetic beads separation. Transfer of the magnetic particles to Washing Plate I.
- **First Washing:** Automatically sample mixing for 1 min. Magnetic beads separation. Transfer of the magnetic particles to Washing Plate II.
- **Second Washing:** Automatically sample mixing for 1 min. Magnetic beads separation. Transfer of the magnetic particles to Washing Plate III.
- **Third Washing:** Automatically sample mixing for 20 s. Magnetic beads separation. 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).

Note: After finishing 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 minute. Transfer the clear supernatant (contains DNA) into a new tube.

Storage of Samples

If you want to…	Then
Continue	Use the eluted DNA directly
Stop	Store the DNA at -25 to -15°C for later analysis

Self-programming of the KingFisher Flex instrument
Protocol information

Protocol name: foodproof_MPK_I_v01
Kit name: foodproof MPK I
Description: KingFisher Flex protocol for isolation of genomic DNA from Gram-negative bacteria from enrichment cultures from raw material and food products.

Plate layouts

Tip Plate		KingFisher 96 KF plate	
Name	Well volume [µL]	Total reagent volume [µL]	Type
-	-	-	-
Lysis Plate		Microtiter DW 96 plate	
Name	Well volume [µL]	Total reagent volume [µL]	Type
Sample	200	-	Sample
Lysis Buffer	320	-	Reagent
Washing Plate 1		Microtiter 96 DW plate	
Name	Well volume [µL]	Total reagent volume [µL]	Type
Wash Buffer I	750	-	Reagent
Washing Plate 2		Microtiter 96 DW plate	
Name	Well volume [µL]	Total reagent volume [µL]	Type
Wash Buffer II	750	-	Reagent
Washing Plate 3		Microtiter 96 DW plate	
Name	Well volume [µL]	Total reagent volume [µL]	Type
Wash Buffer III	750	-	Reagent

Elution Plate		Microtiter 96 DW plate	
Name	Well volume [µL]	Total reagent volume [µL]	Type
Elution Buffer	300	-	Reagent

Dispensed reagents

Lysis Plate		Microtiter DW 96 plate	
Name	Step	Well volume [µL]	Total reagent volume
Binding Buffer	Adjust Binding	315	-

File Steps

Tip 1		**Tip Comb 96 DWH**	
Pick-Up		**Tip Plate**	
Lysis Step		**Lysis Plate**	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	95
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
Adjust Binding		**Lysis Plate**	
		Message	Add Binding Buffer
		Dispensing volume [µl]	315
	Reagent(s)	Name	Binding Buffer
		Volume [µl]	315
Binding		**Lysis 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	4
		Collect time [s]	3
	**Washing_1**	**Washing Plate 1**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	**Washing_2**	**Washing Plate 2**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	**Washing_3**	**Washing Plate 3**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:00:20, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	5
	**Elution**	**Elution Plate**	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium

	Mixing / heating:	Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	90
		Preheat	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	15
	**Bead Removal**	**Washing Plate 3**	
		Release time, speed	00:00:30, Fast
	**Leave**	**Tip Plate**	

3.5 Protocol for the DNA extraction with the foodproof RoboPrep 32 instrument

Additional Equipment and Reagents required

- foodproof RoboPrep 32 instrument
- Pipette and pipette tips
- Disposable gloves
- ddH_2O
- Vortex
- Absolute isopropanol (96-98 %)
- 96 Deep Well Plate
- Tip Comb

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 semi-automated DNA isolation from 200 µL sample material with the **food**proof RoboPrep 32 instrument:

1. Switch on the foodproof RoboPrep 32 instrument.
2. Open the door and push the lever forward, the temperature-controlling module will sink in.
3. Set up the Tip Combs on the mixing sleeve track.

Note: Please read the user manual carefully before starting the purification process with the foodproof RoboPrep 32 instrument! **Resuspend the Lysis Buffer and the magnetic beads in the Binding Buffer thoroughly directly before use!**

4. 96 Deep Well Plate setup and reagent volumes:

Column	Reagent to add	Volume [µL]
1 or 7	Lysis Buffer	320
	Reagent P (if necessary)	25
2 or 8	Wash Buffer I	750
3 or 9	Wash Buffer II	750
4 or 10	Wash Buffer III	750
6 or 12	Elution Buffer	300

5. Transfer **200 µL** of the **sample** into the wells of the 1st or 7th column.
6. Place the 96 Deep Well Plate into the instrument.
7. Select protocol for MPK I.
8. Press  to start the extraction process.
9. After an elevated lysis step of 15 min a pause step occurs. Take out the 96 Deep Well Plate and add **315 µL Binding Buffer** to the wells in the 1st or 7th column. Reinsert the 96 Deep Well Plate into the instrument and press  button to continue with the run. From this point on, the instrument will continue with the purification process without any further user interaction.
10. After finishing the extraction protocol, column 6 or 12 contains the extracted DNA. Transfer the DNA into a 1.5 mL reaction tube and centrifuge at maximum speed for 1 minute. Transfer the clear supernatant (containing DNA) into a new tube.
11. It is recommended to clean the instrument with 70 % ethanol and disinfect it via the UV light program in the apparatus.

The following purification steps will run automatically on the instrument:

- **Lysis of cells:** Cell lysis for 15 min by continuously mixing.
- **Binding of the DNA:** Automatic sample mixing for 15 min. Magnetic beads separation. Transfer of the magnetic particles to Wash Buffer I.
- **First Washing:** Automatic sample mixing for 1 min. Magnetic beads separation. Transfer of the magnetic particles to Wash Buffer II.
- **Second Washing:** Automatic sample mixing for 1 min. Magnetic beads separation. Transfer of the magnetic particles to Wash Buffer III.
- **Third Washing:** Automatic sample mixing for 30 s. 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 15 minutes by continuously mixing. Magnetic beads separation. The magnetic beads will automatically be removed and transferred in Wash Buffer III (disposal).

Storage of Samples

If you want to…	Then
Continue	Use the eluted DNA directly
Stop	Store the DNA at -25 to -15°C for later analysis

Self-programming of the foodproof RoboPrep 32 instrument

Edit and run the experiment program as follow:

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 s)	Temp. (40 to 80°C)	Pause
☒	1	Lysis	0	15	520	3	0	80	☒
☒	1	Binding	0	15	835	3	30	0	☐
☒	1	Binding	0	0	835	0	30	0	☐
☒	2	Wash-1	0	3	750	3	30	0	☐
☒	2	Wash-1	0	0	750	0	30	0	☐
☒	3	Wash-2	0	3	750	3	30	0	☐
☒	3	Wash-2	0	0	750	0	30	0	☐
☒	4	Wash-3	0	1	750	3	30	0	☐
☒	6	Elution	0	15	300	3	60	80	☐
☒	4	Waste	0	1	750	3	0	0	☐

4. Typical Results

4.1 Purity

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.	• Always store the kit at 15 to 25 °C upon arrival.
	Buffer or other reagents were exposed to conditions that reduced their effectiveness.	• Store all buffers 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 -25 to -15 °C.
	Isopropanol not added to foodproof Magnetic Preparation Kit I Binding Buffer, Wash Buffer I or Wash Buffer II.	• Add absolute isopropanol to the foodproof Magnetic Preparation Kit I Binding Buffer, Wash Buffer I and Wash Buffer II before using. • After adding isopropanol, mix the foodproof Magnetic Preparation Kit I Binding Buffer, Wash Buffer I and Wash Buffer II well and store at 15 to 25 °C. • Always mark foodproof Magnetic Preparation Kit I Binding Buffer, Wash Buffer I and Wash Buffer II bottles to indicate the addition of isopropanol.
	Reagents and samples not completely mixed.	• Always mix the sample tube well after the addition of each reagent.
	Not enough target organisms in enrichment culture.	• Prolong the incubation phase.

6. 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 if the product is contaminated by improper storage or handling;

(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.

7. Supplementary Information

7.1 Ordering Information

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

7.2 Trademarks

foodproof® is a trademark of Hygiena Diagnostics GmbH.
Hygiena® is a registered trademark of Hygiena.
JANUS®, WinPREP®, and Varispan™, are trademarks of PerkinElmer, Inc.
KingFisher™ is a trademark of Thermo Fisher Scientific
Other brand or product names are trademarks of their respective holders.

7.3 Contact and 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.

7.4 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 11 L.

8. Change Index

Version 1:
First version of the package insert.
Version 2:
Added note for protein-rich food samples
Deck-Layout foodproof RoboPrep+ 100-8 added.
Warranty and Disclaimer of Liability added
Version 3:
Page 4: Change in volume of reagents
Page 6: Change in volume of added isopropanol
Version 4:
Integration of the RoboPrep Fusion protocol
Integration of the semi-automated protocol for the KingFisher Flex
Version 5:
Integration of the RoboPrep 32 protocol
Version 6:
Rebranding: Change of Logo and Company Name
Revision A:
New document layout, product number.
S 400 11L 20 = INS-KIT230180-REVA

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