For testing of food and environmental samples

foodproof® Magnetic Preparation Kit II

Revision A, July 2023

Application manual for the automated isolation of gram-positive bacterial DNA from enrichment cultures of food samples using the **food**proof® RoboPrep® Series

Product No. KIT230181

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 II 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 II Binding Buffer (Bottle 2), foodproof Magnetic Preparation Kit II Wash Buffer I (Bottle 3) and the foodproof Magnetic Preparation Kit II Proteinase K (Bottle 8) contain irritating compounds that are harmful when brought into 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.

Number of Preparations

480 isolations

Storage

Store the kit at 15 to 25 °C through the expiration date printed on the label.
Once the foodproof Magnetic Preparation Kit II Lysozyme and the foodproof Magnetic Preparation Kit II Proteinase K are dissolved, store aliquots of these solutions at -25 to -15 °C.

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 II Lysis Buffer	• 280 mL • For lysis of cells and extraction of DNA
2	foodproof Magnetic Preparation Kit II Binding Buffer	• 120 mL, add 80 mL absolute isopropanol • For binding of DNA to the magnetic beads
3	foodproof Magnetic Preparation Kit II Wash Buffer I	• 254 mL, add 154 mL absolute isopropanol • For removing impurities
4	foodproof Magnetic Preparation Kit II Wash Buffer II	• 246 mL, add 164 mL absolute isopropanol • For removing impurities
5	foodproof Magnetic Preparation Kit II Wash Buffer III	• 410 mL • For removing impurities
6	foodproof Magnetic Preparation Kit II Elution Buffer	• 192 mL • For elution of DNA
7	foodproof Magnetic Preparation Kit II Lysozyme	• Crystalline • 5 x 11 mg • For digestion of bacterial cell wall
8	foodproof Magnetic Preparation Kit II Proteinase K	• Lyophilizate (freeze-dried) • 3 x 100 mg • For protein digestion and inactivation of endogenous nucleases.

2. How to Use this Product

2.1 Product Overview

Test Principle

The foodproof Magnetic Preparation Kit II, 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. Both, the foodproof RoboPrep+ Series workstation or the foodproof RoboPrep Fusion system perform all steps of the sample preparation procedure and can also perform the PCR setup procedure.

Following concentration by centrifugation, the cells are lysed during a short incubation with the provided foodproof Magnetic Preparation Kit II Lysis Buffer and foodproof Magnetic Preparation Kit II Lysozyme. After addition of the foodproof Magnetic Preparation Kit II Binding Buffer and foodproof Magnetic Preparation Kit II Proteinase K 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 II Lysis Buffer and foodproof Magnetic Preparation Kit II Lysozyme
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 II Elution Buffer

Application

The foodproof Magnetic Preparation Kit II 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-positive bacteria. The quality of the DNA obtained with the kit is highly suitable for applications using any PCR System.

Sample Material

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

Quality Control

- Fraser 1/2 broth [1] spiked with about 5 x 10^4 CFU/mL *Listeria monocytogenes* is extracted and purified as described below.
- 5 µL of the eluate is analyzed using the foodproof *Listeria monocytogenes* Detection Kit (Product No. KIT 2300 48). 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	foodproof Magnetic Preparation Kit II Binding Buffer	Add 80 mL absolute isopropanol to foodproof Magnetic Preparation Kit II 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	foodproof Magnetic Preparation Kit II Wash Buffer I	Add 154 mL absolute isopropanol to foodproof Magnetic Preparation Kit II Wash Buffer I. **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.
4	foodproof Magnetic Preparation Kit II Wash Buffer II	Add 164 mL absolute isopropanol to foodproof Magnetic Preparation Kit II 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.
7	foodproof Magnetic Preparation Kit II Lysozyme	Dissolve foodproof Magnetic Preparation Kit II Lysozyme in 1.1 mL double-distilled water; aliquot solution.	Store at -25 to -15 °C. Stable for 12 months.
8	foodproof Magnetic Preparation Kit II Proteinase K	Dissolve foodproof Magnetic Preparation Kit II Proteinase K in 5 mL double-distilled water; aliquot solution.	Store at -25 to -15 °C. Stable for 12 months.

3.2 Protocol for foodproof RoboPrep⁺ workstation

The foodproof Magnetic Preparation Kit II Lysozyme is added to the foodproof Magnetic Preparation Kit II Lysis Buffer in the following concentration. Add the appropriate volume of the foodproof Magnetic Preparation Kit II Lysozyme to the foodproof Magnetic Preparation Kit II Lysis Buffer based on the number of reactions/samples to be processed.

Note: Always prepare the mixture for 10 additional reactions/samples due to death volume in the reagent reservoir of the robotic workstation and pipetting losses. After the start of the foodproof Roboprep⁺ workstation, the necessary volume of the two reagents for the given numbers of samples will be displayed.

Number of reactions/samples	Components in mL	
	foodproof Magnetic Preparation Kit II Lysis Buffer	Lysozyme
1	0.49	0.01
5	2.45	0.05
10	4.90	0.10
15	7.35	0.15
20	9.80	0.20
25	12.25	0.25
30	14.70	0.30
35	17.15	0.35
40	19.60	0.40
45	22.05	0.45
50	24.50	0.50
55	26.95	0.55
60	29.40	0.60
65	31.85	0.65
70	34.30	0.70
75	36.75	0.75
80	39.20	0.80
85	41.65	0.85
90	44.10	0.90
95	46.55	0.95
100	49.00	1.00
105	51.45	1.05
110	53.90	1.10
115	56.35	1.15
120	58.80	1.20

The foodproof Magnetic Preparation Kit II Proteinase K is added to the foodproof Magnetic Preparation Kit II Binding Buffer in the following concentration. Add the appropriate volume of the foodproof Magnetic Preparation Kit II Proteinase K to the foodproof Magnetic Preparation Kit II Binding Buffer depending on the number of reactions/samples to be processed.

Note: Always prepare the mixture for 10 additional reactions/samples due to death volume in the reagent reservoir of the robotic workstation and pipetting losses. After the start of the foodproof RoboPrep^{+} workstation, the necessary volume of the two reagents for the given numbers of samples will be displayed.

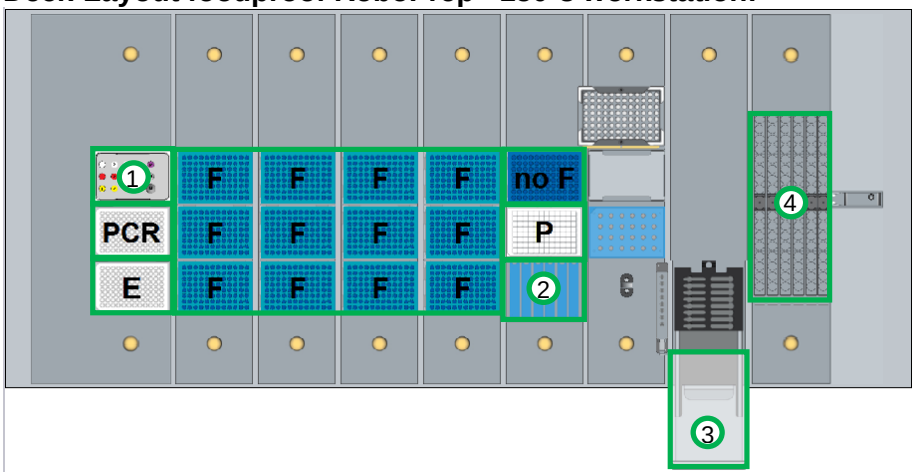
Number of reactions/samples	Components in mL	
	foodproof Magnetic Preparation Kit II Binding Buffer	Proteinase K
1	0.315	0.025
5	1.575	0.125
10	3.150	0.250
15	4.725	0.375
20	6.300	0.500
25	7.875	0.625
30	9.450	0.750
35	11.025	0.875
40	12.600	1.000
45	14.175	1.125
50	15.750	1.250
55	17.325	1.375
60	18.900	1.500
65	20.475	1.625
70	22.050	1.750
75	23.625	1.875
80	25.200	2.000
85	26.775	2.125
90	28.350	2.250
95	29.925	2.375
100	31.500	2.500
105	33.075	2.625
110	34.650	2.750
115	36.225	2.875
120	37.800	3.000

Additional Equipment and Reagents required

- foodproof RoboPrep+ workstation
- RAININ disposable filter tips, 250 µL
- RAININ disposable tips, 1,000 µL
- RAININ disposable filter tips, 1,000 µL
- Eppendorf troughs (reagent holders)
- Process deep well plates
- Elution micro plates
- 5 mL tubes (master mix preparation)
- Disposable waste bags
- 12 mL sample tubes
- PCR reagents
- PCR plates
- 96-cap cover pads for deep well plate
- Isopropanol, absolute
- Water, double-distilled
- Centrifuge for deep well plates capable of a 2,250 × g centrifugal force

Placement of Reagents and Equipment

Deck-Layout foodproof RoboPrep+ 150-8 workstation:



Deck-Layout foodproof RoboPrep+ 100-8 workstation:

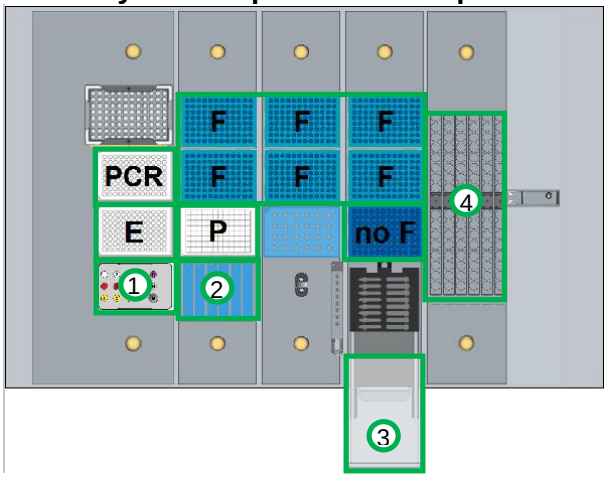


Figure 1. Positioning of reagents and equipment on the deck of the foodproof **RoboPrep+ workstations**

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 pickup 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).

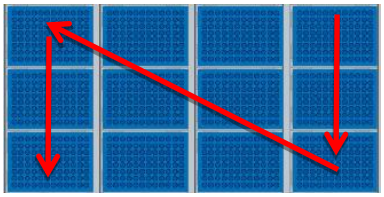


Figure 2. Directions for placing tips on the deck

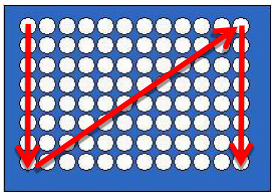


Figure 3. Tip pickup direction within tip racks

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).

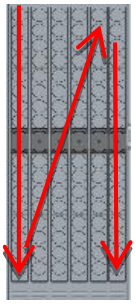


Figure 4. Directions for positioning sample tubes

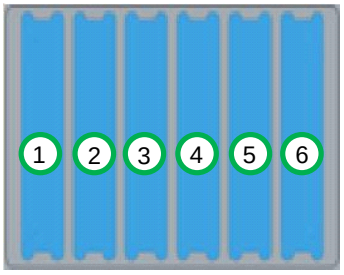
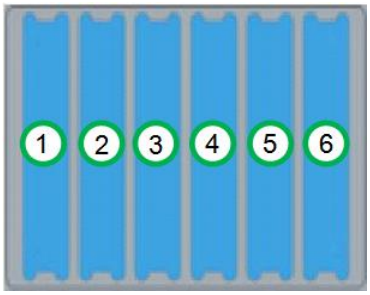


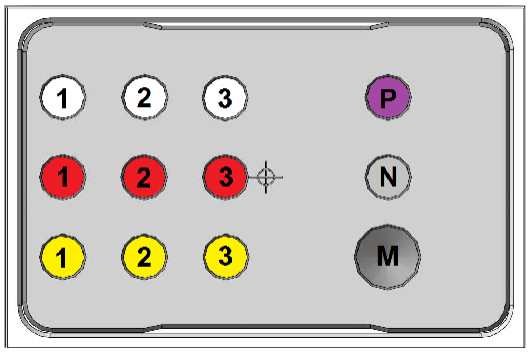
Figure 5. Reagent containers

5. **Load the reagent containers with the following kit components (2, Fig. 5)**



1: Lysis Buffer + Lysozyme
2: Binding Buffer + Proteinase K
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., always wear a lab coat). 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 0.5 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 login screen will be displayed (Fig. 7).**

Figure 7. Lirix3 login 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.

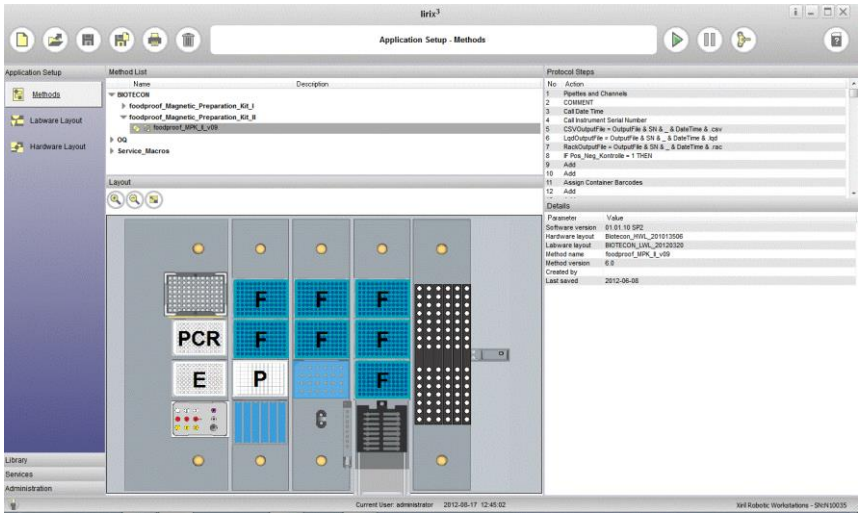


Figure 8. Application Setup - Methods window.

8. **Select method "foodproof_MPK_II_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.

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

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

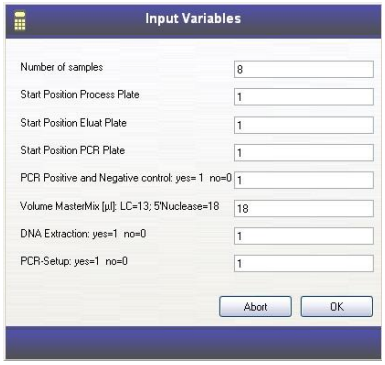


Figure 9. Input Variables - Dialog Window

10. **Choose the number of samples: 1 – 96.**
11. **Choose the start position for the Process Plate, the Eluate Plate and the PCR Plate.**
12. **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.**
13. **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.
14. **Choose whether you want to conduct a DNA extraction.**

15. **Choose whether you want to conduct a PCR setup. Click OK to continue.**

16. **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 II 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. Volume Lysis Buffer - Dialog Window

22. **Check for the necessary volume of Lysyzyme (Fig. 12).**

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

Figure 12. Volume Lysozyme - Dialog Window

23. **Check for the necessary volume of Binding Buffer (Fig. 13).**

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

Figure 13. Volume Binding Buffer - Dialog Window

24. Check for the necessary volume of Proteinase K (Fig. 14).

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

Figure 14. Volume Proteinase K - Dialog Window

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

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

Figure 15. Volume Wash Buffer I - Dialog Window

24. Check for the necessary volume of Wash Buffer II (Fig. 16).

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

Figure 16. Volume Wash Buffer II - Dialog Window

25. Check for the necessary volume of Wash Buffer III (Fig. 17).

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

Figure 17. Volume Wash Buffer III- Dialog Window

26. Check for the necessary volume of Elution Buffer (Fig. 18).

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

Figure 18. Volume Elution Buffer - Dialog Window

27. Check for the necessary plates (Fig. 19).

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

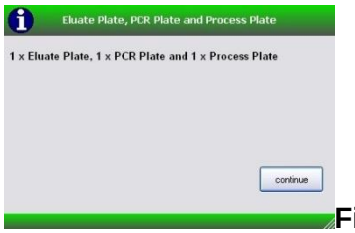


Figure 19. Requested plates - Dialog Window

28. Check for the necessary number of racks with filter tips (Fig. 20).

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 20. Racks with filter tips- Dialog Window

29. Check for the necessary number of tips without filters (Fig. 21).

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

Figure 21. Number of tips without filter- Dialog Window

30. Check for the PCR reagents (Fig. 22).

Place the necessary PCR reagents (Internal Control, Enzyme Solution and Master Mix) into the PCR setup rack and confirm with "continue".

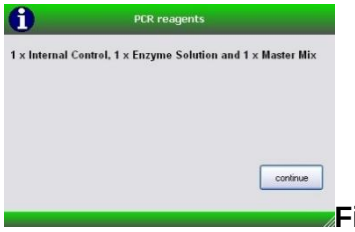


Figure 22. PCR reagents- Dialog Window

31. Check for the PCR Positive and Negative Control (Fig. 23)

Place the Positive and Negative Control into the PCR setup rack and confirm with "continue".

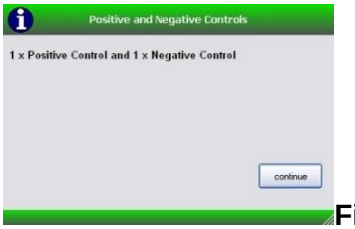


Figure 23. PCR Control reagents- Dialog Window

32. Check for the 5 mL tube for the Master Mix preparation (Fig. 24).

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

Figure 24. Tube for Master Mix- Dialog Window

33. Centrifugation of the Process Plate (Fig. 25)

After the 0.5 mL sample material was transferred into the process plate, take the process plate out of the robotic workstation, seal the plate with a 96-cap cover pad and centrifuge the plate for 10 min at minimum 2,250 x g.

Afterwards, place the process plate without cover pad back at the start position and confirm with "continue".

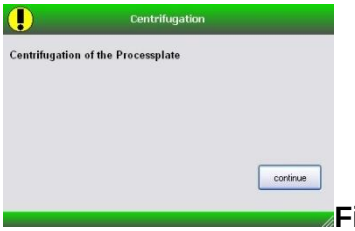


Figure 25. Centrifugation step- Dialog Window

34. All next steps are fully automated, and a software message on the screen will indicate when the protocol is finished (Fig. 26).

Figure 26. 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.

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
- 96 cap cover pads for deep well plate
- 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:

1. Daily Preventative Maintenance

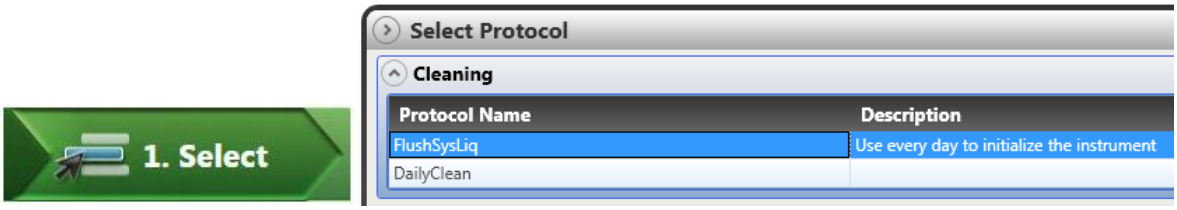
Flushing the Varispan™ system of the **food**proof RoboPrep Fusion with degassed distilled water helps to keep the system free of air bubbles, crystals, precipitates, and biological growth that can accumulate within the tubing, valves, and syringes. If allowed to accumulate in the liquid path,

these items decrease the accuracy and precision of the instrument. To prevent this problem, **flush the system at the start of the working day**.

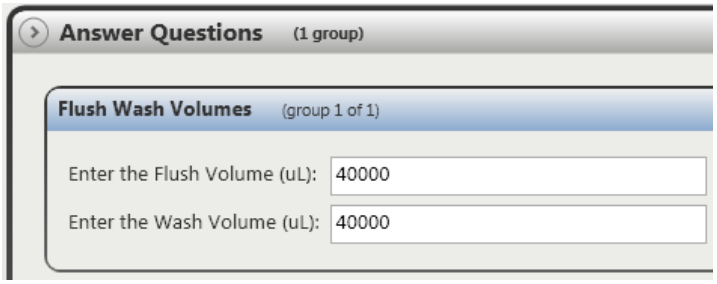
To flush the system:

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

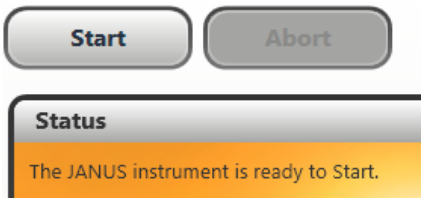
2. Click on the Select button and choose the protocol "FlushSysLiq" under the Select Protocol section "Cleaning" of the window to start the flush process.



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



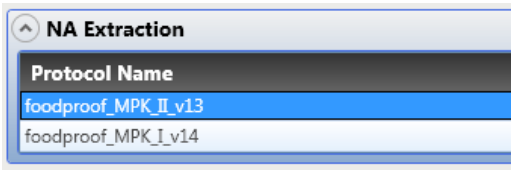
4. Click on the Run button and the Start button to start the process.



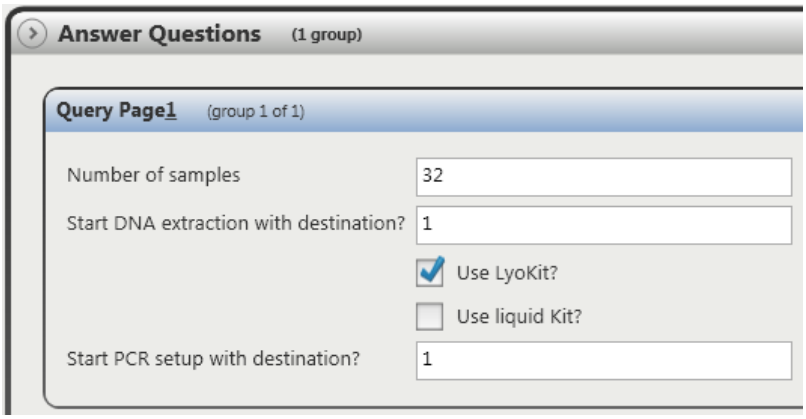
5. While the protocol is running, the relative status of the protocol is constantly updated on the screen by the Progress panel.

B. Executing the foodproof MPK II protocol

1. Click on the Select button and choose the protocol "foodproof_MPK_II_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:

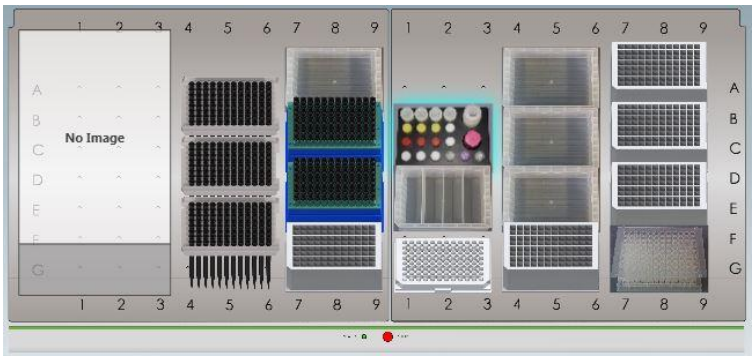
4. Inspect the checklist (under the section Gather the Following Labware and Reagents) and collect the labware that you will need to run the protocol. Click on each labware item that you collect. All labware that is listed is required. For your convenience, the location of a lab item or reagent may be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

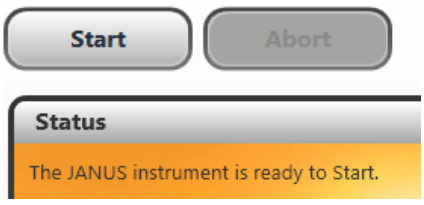
7. When you select an item in the top left section of the window 'Place the Following Labware and Reagents', the item's placement instructions are displayed under the Instructions section of the window:

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

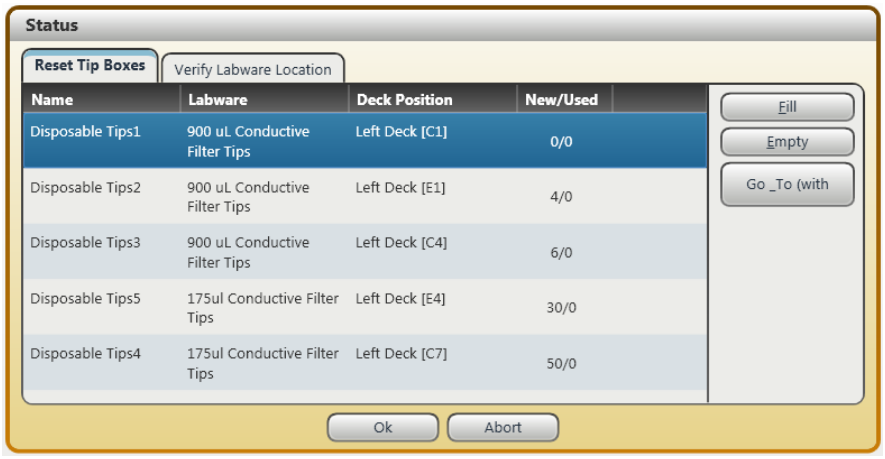


8. Pipet 5 – 10 mL of the food enrichment culture into a 12 mL sample tube and place it into the sample tube rack.

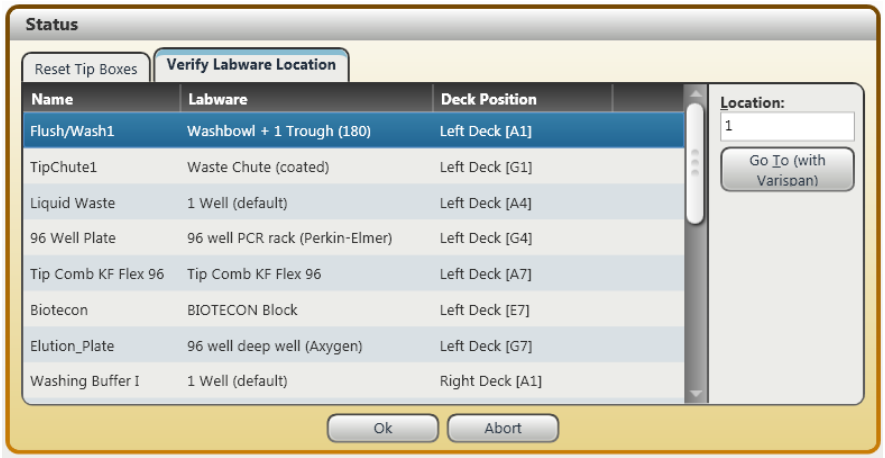
9. Run the protocol and monitor its progress as the protocol executes.



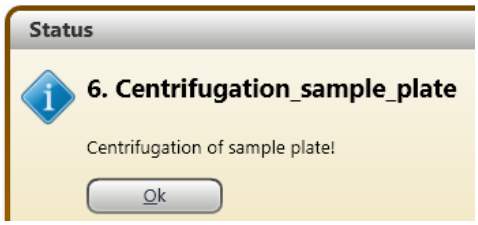
10. Reset tip boxes or start the tips remaining from a previous run.



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

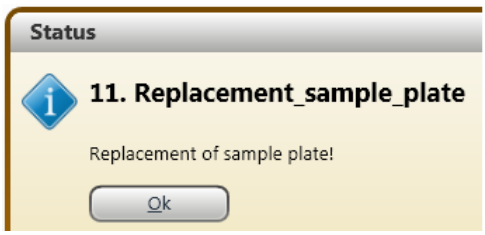


12. Centrifugation step after sample transfer.

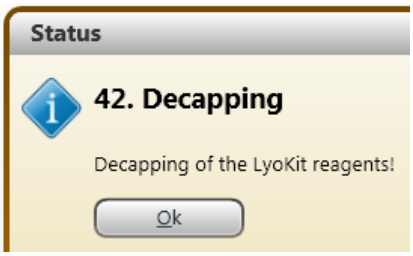


Seal the Lysis Plate with a 96-cap cover pad and centrifuge the plate for 10 min at minimum 2,250 x g.

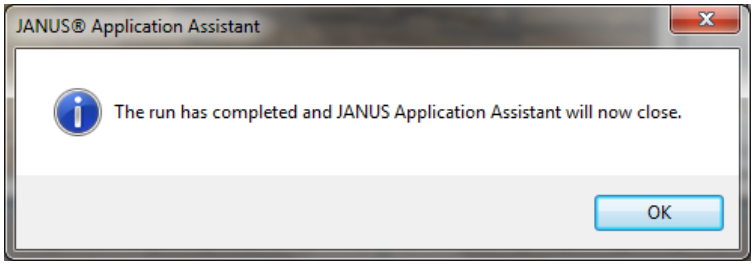
13. Replace the sample plate on the PE JANUS deck.



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



15. The following window indicates that the protocol is finished



16. Cleanup 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 0.5 mL enrichment culture with the foodproof RoboPrep Fusion:

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

   Elution Plate: 200 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
2. Transfer of 500 µL sample into the Lysis Plate.
3. Instrument paused to conduct the centrifugation of the Lysis plate.
4. Seal the Lysis Plate with a 96-cap cover pad and centrifuge the plate for 10 min at minimum 2,250 x g.
5. Replace the Lysis plate and continue the process.
6. Pipetting and discarding the supernatant out of the Lysis Plate.
7. Lysis Plate: 490 µL Lysis Buffer, and 10 µL Lysozyme added.
8. Transport of the KingFisher Flex plates to the KingFisher Flex instrument.
9. Execution of the foodproof MPK II program on the KingFisher Flex instrument.
10. After an elevated lysis step of 10 minutes a pause step occurs. The Lysis Plate is automatically transported to the PerkinElmer JANUS G3 instrument.
11. Lysis Plate: 315 µL Binding Buffer and 25 µL Proteinase K added.
12. Transport of the Lysis plate to and continuation of the program on the KingFisher Flex instrument.
13. After finishing the extraction protocol, the Elution Plate containing the extracted DNA and the other plates are transported back to the PerkinElmer® JANUS® instrument.
14. Execution of the PCR setup in the PerkinElmer 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 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 KingFisher Flex instrument

Protocol information

Protocol name: foodproof_MPK_II_v01
Kit name: foodproof MPK II
Description: KingFisher Flex protocol for isolation of genomic DNA from Gram-positive 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	50	-	Sample
Lysis Buffer	500	-	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	200	-	Reagent

Dispensed reagents

Lysis Plate		Microtiter DW 96 plate	
Name	Step	Well volume [µL]	Total reagent volume
Binding Buffer	Adjust Binding	315	-
Reagent P	Adjust Binding	25	-

File Steps

	Tip 1	**Tip Comb 96 DWH**	
	Pick-Up	**Tip Plate**	
	Lysis Step 1	**Lysis Plate**	
	Beginning of step	Pre-collect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	55
		Preheat	Yes
	End of step	Post mix	No
		Collect beads	No
	Adjust Binding	**Lysis Plate**	
		Message	Add Binding Buffer and Reagent P
		Dispensing volume [µL]	340
	Reagent(s)	Name	Binding Buffer
		Volume [µL]	315
		Name	Reagent P
		Volume [µL]	25
	Pause 1	**Lysis Plate**	
		Message	Wait for Flex
	Lysis Step 2	**Lysis Plate**	
	Beginning of step	Pre-collect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating temperature [°C]	95
		Preheat	Yes
	End of step	Post mix	No
		Collect beads	No
	Binding	**Lysis Plate**	
	Beginning of step	Pre-collect	No

		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Post mix	No
		Collect count	4
		Collect time [s]	3
	**Washing_1**	**Washing Plate 1**	
	Beginning of step	Pre-collect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Post mix	No
		Collect count	4
		Collect time [s]	5
	**Washing_2**	**Washing Plate 2**	
	Beginning of step	Pre-collect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Post mix	No
		Collect count	4
		Collect time [s]	5
	**Washing_3**	**Washing Plate 3**	
	Beginning of step	Pre-collect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:00:20, Fast
		Heating during mixing	No
	End of step	Post mix	No
		Collect count	3
		Collect time [s]	5

	Elution	**Elution Plate**	
	Beginning of step	Pre-collect	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	Post mix	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**	

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 II Binding Buffer, Wash Buffer I or Wash Buffer II.	• Add absolute isopropanol to the foodproof Magnetic Preparation Kit II Binding Buffer, Wash Buffer I and Wash Buffer II before using. • After adding isopropanol, mix the foodproof Magnetic Preparation Kit II Binding Buffer, Wash Buffer I and Wash Buffer II well, and store at 15 to 25 °C. • Always mark foodproof Magnetic Preparation Kit II Binding Buffer, Wash Buffer I and Wash Buffer II bottle to indicate the addition of isopropanol.
	Reagents and samples not completely mixed.	• Always mix the sample tube well after addition of each reagent.
	Not enough target organisms in enrichment culture.	• Prolong the incubation phase.

6. References

1. Fraser, J.A. and Sperber, W.H. (1988) *J.Food Protect.* 51, Nr. 10, 762-765.

7. 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.

8. Supplementary Information

8.1 Ordering Information

Hygiena® is offering 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 trademark of Hygiena Diagnostics GmbH.
Hygiena® is a registered trademark of Hygiena.
JANUS®, WinPREP®, and Varispan™, are trademarks of PerkinElmer, Inc.
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8.3 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.

8.4 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 12 L.

9. Change Index

Version 1:
First version of the package insert.

Version 2:
Page 5: NOTE for protein-rich food samples
Page 8: Deck-Layout foodproof RoboPrep+ 100-8 added.
Page 20: 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 KF Flex

Version 5:
Integration of the RoboPrep 32 protocol

Version 6:
New document layout and content. New order number.

Revision A (Version 5)
New branding and document layout.
S 400 12 L 20 = INS-KIT230181-REVA

Hygiena LLC
Camarillo, CA 93012
USA
diagnostics.support@hygiena.com

Manufactured by
Hygiena Diagnostics GmbH

Hermannswerder 17
14473 Potsdam
Germany

www.hygiena.com

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