foodproof® Magnetic Preparation Kit II

Order No. S 400 12 L

Quick Reference Procedure

Version 2, October 2013

The **food**proof Magnetic Preparation Kit II in combination with the **food**proof RoboPrep+ Series workstation provides fully automated purification of total genomic bacterial DNA from enrichment cultures of various food samples (raw material and processed food) and is optimized for Gram-positive bacteria. The DNA is suitable for direct use in PCR applications. Following concentration by centrifugation, the cells are lysed during a short incubation with the Lysis Buffer and Lysozyme. After addition of the Binding Buffer and the Proteinase K the DNA selectively binds to the magnetic beads. Bound DNA is purified in three washing steps. The elution buffer releases the DNA from the magnetic beads.

A. Preparation of Kit Working Solutions

In addition to the ready-to-use solutions supplied with the kit, preparation of working solutions is required:

Bottle	Content	Preparation of working solution
No. 2 (green cap)	Binding Buffer	Add 80 ml absolute isopropanol
No. 3 (blue cap)	Wash Buffer I	Add 154 ml absolute isopropanol
No. 4 (blue cap)	Wash Buffer II	Add 164 ml absolute isopropanol
No. 7 (white cap)	Lysozyme	Dissolve Lysozyme in 1.1 ml double- distilled water
No. 8 (purple cap)	Proteinase K	Dissolve Proteinase K in 5 ml double- distilled water

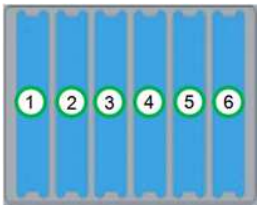
Check the box on the label of the bottle after isopropanol has been added. Add the date for verifiability. Store at 15 – 25 °C. Stable until the expiry date printed on kit label, respectively stable for 12 months (Lysozyme and Proteinase K).

B. Additional Equipment and Reagents

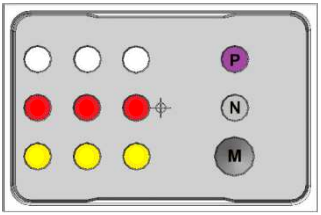
All necessary plastic consumables are available through BIOTECON Diagnostics in a package (Order No. Z 600 01) for 960 reactions. The 96 cap cover pads for deep well plate are separately available (Order No. Z 100 29).

C. Placement Procedure

1. Place the PCR plate, the elution micro plate and the process deep well plate at the appropriate starting positions.
2. Place the disposable waste bag at the appropriate position.
3. Load the metal racks with tips (1000 µl).
4. Load the sample tubes into the rack.
5. Load the reagent containers and PCR setup rack with the following kit components.



1: Lysis Buffer + Lysozyme
2: Binding Buffer + Proteinase K
3: Wash Buffer I
4: Wash Buffer II
5: Wash Buffer III
6: Elution Buffer



White: Internal Control
Red: Enzyme Solution
Yellow: Master Mix
P: Control Template
N: Negative Control: PCR-grade H_2O
M: 5 ml tube for PCR Mix

D. Protocol: Purification of Total DNA from 500µl Food Enrichment Culture

1. Place **5 – 10 ml** of food enrichment culture into 12 ml **sample tubes.**

2. Switch on the **food**proof RoboPrep+ **workstation** and monitor. Allow the system to boot up.
3. Double-click the **lirix3 shortcut icon** on the desktop.
4. Enter **user name** and **password** on log-in screen. Note that a password was set for all users in the access control manager. Select OK.
5. Select **method "foodproof_MPK_II_vxx**" in the displayed screen "Application Setup – Methods". Start the method by clicking on the run button.
6. Select **input variables** from the "Input Variables" dialog window.
7. Choose the **number of samples: 1 – 96**.
8. Choose the **start position** for the Process Plate, the Eluate Plate and the PCR Plate.
9. For **PCR setup**, choose whether a positive and a negative control should be included.
   YES: PCR mastermix will be prepared for one positive and one negative control each in addition to the number of samples.
   NO: PCR mastermix will be prepared for the processed number of samples only.
10. 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.**
11. Choose whether you want to conduct a **DNA extraction**.
12. Choose whether you want to conduct a **PCR setup**. Click OK to continue.
13. In the "Start Run" dialog window, 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. 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.
14. The **instrument is initialized** and the run starts.
15. Place the **sample tubes** into the **sample tube racks**.

Request for all necessary equipment and reagents: The following dialog windows will guide you through the remaining steps required to set up the RoboPrep+ workstation for the "**food**proof Magnetic Preparation Kit II Process".

16. Check the necessary volume of **Lysis Buffer** and fill it into the **first reagent reservoir**. Confirm with "continue".
17. Check the necessary volume of **Lysozyme** and add it to the **first reagent reservoir**. Confirm with "continue".
18. Check the necessary volume of **Binding Buffer** and fill it into the **second reagent reservoir**. Confirm with "continue".
19. Check the necessary volume of **Proteinase K** and add it to the **second reagent reservoir**. Confirm with "continue".
20. Check the necessary volume of **Wash Buffer I** and fill it into the **third reagent reservoir**. Confirm with "continue".
21. Check the necessary volume of **Wash Buffer II** and fill it into the **fourth reagent reservoir**. Confirm with "continue".
22. Check the necessary volume of **Wash Buffer III** and fill it into the **fifth reagent reservoir**. Confirm with "continue".
23. Check the necessary volume of **Elution Buffer** and fill it into the **sixth reagent reservoir**. Confirm with "continue".
24. Check the necessary plates**.** Place the **elution plate**, the **PCR plate** and the **process plate** at the start positions. Confirm with "continue".
25. Check the necessary **number of racks with filter tips** and place them on the deck beginning with the first tip rack defined in the labware layout. Confirm with "continue".
26. Check the necessary number of **tips without filters** and place them into the defined rack. Confirm with "continue".
27. Check the **PCR reagents** and place these into the PCR setup rack. Confirm with "continue".
28. Check the PCR **Positive and Negative Control** and place them into the PCR setup rack. Confirm with "continue".
29. Check the **5 ml tube** for the **Master Mix preparation** and place it into the PCR setup rack. Confirm with "continue".
30. **Centrifugation of the Process Plate:** After the sample material was transferred into the process plate, take it 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. Confirm with "continue".

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

*** Note:** for further information please refer to: www.bc-diagnostics.com

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