foodproof® Magnetic Preparation Kit I Order No. S 400 11 L

Quick Reference Procedure for KingFisher® Flex Instrument

Version 1, March 2018

The **food**proof Magnetic Preparation Kit I in combination with the KingFisher® Flex workstation provides semi-automated purification of bacterial DNA from up to 200 µl enrichment culture of food samples (raw material and processed food). The DNA isolation process is based on magnetic bead technology, which relies on the interaction of nucleic acids with coated magnetic particles under suitable buffer conditions. The kit provides high-quality DNA, which is suitable for direct use in PCR applications.

A. Preparation of Kit Working Solutions

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

Bottle/Tube	Content	Preparation of working solution
No. 2 (green cap)	Binding Buffer	Add 80 ml absolute isopropanol to one bottle of Binding Buffer
No. 3 (blue cap)	Wash Buffer I	Add 154 ml absolute isopropanol to one bottle of Wash Buffer I
No. 4 (blue cap)	Wash Buffer II	Add 164 ml absolute isopropanol to one bottle of Wash Buffer II

Check the box on the label of the bottle after isopropanol has been added. Add the date for verifiability.
All buffers and kit components of the **food**proof Magnetic Preparation Kit I should be stored at 15 °C to 25 °C and are stable through the expiration date printed on the label.

B. Additional Equipment and Reagents

For protein-rich food samples (e.g. egg, pork, chicken, salmon, cheese), addition of Reagent P (Order No. A 500 12) to the Lysis Buffer is necessary.
All necessary plastic consumables are available through BIOTECON Diagnostics.

- KingFisher® Flex instrument
- Pipette and pipette tips
- Disposable gloves
- ddH_2O
- Vortexer
- absolute isopropanol (96-98 %)
- Deep well plates, 2.0 ml (Order No. Z 100 54)
- KingFisher 96 KF plate, 200 µl (Order No. Z 100 55)
- Tip Comb 96 DWH (Order No. Z 100 53)
- Adhesive Seal (Order No. Z 100 61)

D. Protocol: Isolation of bacterial DNA from 200 µl sample material with the KingFisher® Flex

1. Switch on the KingFisher® Flex instrument.

Note: *Before starting the purification process with the KingFisher® Flex instrument please read carefully the user manual!*
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**" 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 min 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…
1. Continue : use eluted DNA directly
2. Stop : store eluted DNA for one week at 4 to 8 °C, or freeze at – 20 °C for long-term storage.

For further information please refer to: www.bc-diagnostics.com

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