



## **USER GUIDE**

for

# Automated purification of PCR products with

KingFisher 96 instrument and Invitrogen ChargeSwitch PCR Clean-Up Kit

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## Descripition

Purification of PCR product from salts, primers, dNTP's and other non-nucleic acid reagents with Invitrogen's ChargeSwitch® PCR Clean-Up Kit beis easily automated using can easily **KingFisher**® instruments (Thermo Fisher Scientific). The KingFisher platforms utilize patented technology where magnetic rods move particles through the processing steps. KingFisher 96 instrument operates on microplates and can process up to 96 samples per run. The instrument can handle volumes up to 1 ml.

DNA fragments ranging in size from 90 bp -40 kb are purified with >80% recovery.

The protocol described here is designed for general use and can be modified according to individual needs using KingFisher<sup>®</sup> Software provided with the instrument.

#### Important notes

- See ChargeSwitch PCR Clean-Up Kit user manual for reagent storage, product use limitations, safety information etc.
- Resuspend ChargeSwitch magnetic beads (DNA Binding Beads) thoroughly before use.

## Importing protocols from the web

KingFisher Software protocol for Invitrogen ChargeSwitch PCR Clean-Up Kit can be downloaded from the website (<u>www.thermofisher.com/kingfisher</u>). First you have to save the file "IVGN\_PCRClean\_KF96" to your computer.

- 1. Open KingFisher Software.
- 2. Select Protocol  $\rightarrow$  Import/Export data.
- 3. Click Read file.
- 4. Select the database (\*.KF2) by browsing in the **Open** dialog and click **Open**.
- 5. Select the protocol(s) you wish to import from the *Protocols in file* list. Use the SHIFT key together with the mouse button to select protocols between two clicked protocols and the CTRL key to select only the clicked protocols.
- 6. Tick **Update existing** if you wish to overwrite the protocols with identical protocol name(s) in the target database.
- 7. Click Import.

If there are protocols with identical names and you have not ticked the **Update existing** tick box, you will be prompted to change the name of the protocol that is being imported:

- Type in a new name and click OK.
  - **Note:** Check that the name of the protocol does not exceed 17 characters.
- You will receive a message stating whether the database updating procedure was successful or not.

## Sending or running a protocol

To send a protocol to the instrument memory, or to run the protocol directly without saving it to the instrument memory:

- 1. Check that the instrument has been configured correctly and that the instrument is connected to the correct COM port.
- 2. Open the Transfer protocol to instrument dialog from Instrument → Send Protocol to Instrument...
- 3. Select the target instrument from the *Select instrument* table.
- 4. Select a protocol from the *Protocols* for *selected instrument* table. The protocols in that table are the available protocols in the database.
- 5. Select either of the following:
  - a. Click **Send protocol** to transfer protocols to the instrument memory.
    - You can launch the protocol using the instrument keypad and display.
  - b. Click **Execute protocol** to launch the protocol directly without transferring it to the instrument memory
    - The protocol will launch after validation.





## KingFisher 96 protocol

#### Sample preparation

- KingFisher protocol with the ChargeSwitch PCR Clean-Up Kit is designed to purify PCR products from salts, primers, dNTP's and other non-nucleic acid reagents
- Use KingFisher Microtiter<sup>™</sup> deepwell microplate (Catalog No. 95040450), KingFisher deepwell tip comb (Catalog No. 97002534) and KingFisher 96 KF plate (Catalog No. 97002540) with ChargeSwitch PCR Clean-Up protocol.
- Add sample and other reagents to KingFisher KF96 plates and start the IVGN\_PCRClean\_KF96 process.

#### **KingFisher 96 process**

**Table 1** Pipetting instructions for KingFisher 96

 and ChargeSwitch PCR Clean-Up protocol.

Plate *	Plate	Content	Sample/ Reagent volume
		PCR Sample	50 µl
А	1	ChargeSwitch Magnetic Beads	10 µl
		Purification buffer (N5)	60 µl
А	2	Wash buffer (W12)	150 µl
A	3	Wash buffer (W12)	150 µl
A	4	Elution Buffer (E5)	50 µl

\* A=KingFisher 96 KF plate

- 1. Combine 50 μl of PCR Sample, 10 μl of resuspended ChargeSwitch Magnetic Beads and 60 μl of Purification Buffer (N5) to plate **1**.
- 2. Add 150 μl of ChargeSwitch Wash Buffer (W12) to plates **2 and 3.**
- 3. Add 50 µl of ChargeSwitch Elution Buffer (E5) to plate **4.**
- 4. Combine the tip comb and the KingFisher plate. See KingFisher 96 User manual.
- 5. Select the ChargeSwitch PCR Clean-Up protocol using arrow keys from the instument and press START button OR execute protocol using KingFisher software.
- 6. Load the plates according to protocol request and press START after every plate to confirm the action.
- 7. **Note!** Confirm that the plates are placed in correct orientation: A1 well to be pointed to upper right corner of the plate holder in

turntable. A1 row of the plate is then always located in the inner circle of the turntable.

- 8. The purification protocol will start when the last plate is loaded and START button is pressed.
- 9. After the purification process is completed, the plates are removed according to instructions shown in instrument screen. Press START after each plate removal to confirm the action.
- 10. When the last plate is removed, the text End\_of\_run will appear. Press STOP to complete the run.

# Description of ChargeSwitch PCR Clean-Up protocol with KingFisher 96

- 1. PCR Sample is incubated first with magnetic beads and Puritication Buffer (N5) in plate 1 for 2 minutes. Magnetic bead/DNA complexes are formed.
- 2. Magnetic beads are washed with ChargeSwitch Wash Buffer (W12) in plates 2 and 3.
- 3. Purified PCR product is released to ChargeSwitch Elution Buffer (E5) in plate4 for 6 minutes.
- 4. Beads are discarded into plate 3.





## Troubleshooting

- Magnetic particles remain in the sample well
   If starting material is too viscose, the magnetic rods will not be able to collect the particles. Dilute the sample.
- 2. Magnetic particles are attached to the tip combs
  - This will not affect the yield because the sample has been released from the particles.
- 3. The volumes of the reagent in one well exceeds the limit.
  - It is recommended to keep the given volumes within certain limits to avoid failure in performance of the chemical reactions and the processor.
- 4. Any steps of the protocol (e.g. sample incubation and elution times) and the reagent volumes can be modified with KingFisher® software.
- The processor is not working properly
   ➢ Refer to Kingfisher 96 User Manual
- 6. If you have questions related to ChargeSwitch chemistry see ChargeSwitch PCR Clean-Up Kit User Manual for detailed troubleshooting instructions.

## **Ordering Information**

Product no.	Product Description		
Thermo Fisher Scientific			
540 05 00	KingFisher 96, 110V-240V, Magnetic particle processor		
24073430	KingFisher 96 head for Deep Well plate		
97002534	KingFisher 96 tip comb for DW magnets (10 x 10 pcs/box)		
97002540	KingFisher 96 KF plate (200 µl), 48 plates/box		
Invitrogen			
CS12000	ChargeSwitch PCR Clean-Up kit (100 preps)		
CS12000-10	ChargeSwitch PCR Clean-Up kit (960 preps)		

## **Contact information**

# Thermo Fisher

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