

KingFisher®

**Automated purification of histidine tagged proteins
with KingFisher® instruments**

INSTRUCTIONS FOR USE

20/10/2003

Description

The histidine tagged recombinant protein purification using Dynabeads® TALON™ can easily be automated using KingFisher® instruments (Thermo Electron Corporation) which utilize patented technology where beads are transferred during processing with magnetic rods. KingFisher mL instrument, suitable for lower throughput need, can process 15 samples per run. KingFisher 96 is designed for higher throughput needs and enables processing up to 96 samples per run.

The protocols described here are designed for general use and can be modified according to customer individual needs using KingFisher® Software provided with the instrument.

The isolated proteins can either be eluted from magnetic particles or remain attached to particle surface for further applications. Two separate protocols for KingFisher mL and KingFisher 96 instruments are provided to enable both these applications (tables 1 and 3).

Buffer recommendations

The following buffers are recommended for use with Dynabeads TALON™ in KingFisher purification process. Alternative binding and/or washing buffers may also be used for isolation of your specific recombinant protein.

TALON™ Binding and Washing Buffer

50 mM NaP, pH 8.0
300 mM NaCl
0.01% Tween 20

TALON™ Elution Buffer

150 mM Imidazole
50 mM NaP pH 8.0
300 mM NaCl
0.01% Tween 20

TALON™ Low pH Elution Buffer

50 mM NaAc, pH 4.5
300 mM NaCl
0.01% Tween 20

Sample preparation recommendations

There are different ways to prepare a cell lysate containing histidine-tagged proteins. The following methods are recommended for KingFisher process. See additional information from Dynabeads TALON package insert.

TALON™ Binding and Washing Buffer supplemented with detergent cocktail

Cell pellet from 1 ml bacterial culture*
590 µl TALON™ Binding and Washing Buffer
10 µl PopCulture Reagent (Novagen)
1 µl DNase I (100 µg/ml)

*For multiple samples (e.g. 96 samples in deepwell plate) you can prepare a lysis buffer master mix.

Alternative lysis methods for *E. coli*

- TALON™ Binding and Washing Buffer with 1% Triton-X-100
- Commercially available ready-made lysis buffers
- French Press
- Sonication

Efficiency of lysis can be increased by addition of lysozyme. To avoid a sticky pellet, the addition of DNase I is recommended.

KingFisher 96

KingFisher 96 protocols

Table 1. KingFisher 96 protocols for His-tagged protein purification

Instrument	Protocol name	Protocol description
KingFisher 96	96_His_tag_1	Used for purification of His-tagged proteins from <i>E. coli</i> lysates. Purified proteins are released from the magnetic particles into the TALON Elution Buffer.
KingFisher 96	96_His_tag_2	Used for purification of His-tagged proteins from <i>E. coli</i> lysates. Magnetic particles with purified proteins attached are released to TALON Binding and Washing Buffer.

Magnet and plastics requirements

The KingFisher 96 protocols 96_His_tag_1 and 96_His_tag_2 are designed to operate with deep well and KingFisher 96 plates (Thermo Electron). Be sure that the appropriate magnet head is installed before starting. If magnet head needs to be changed follow the KingFisher 96 User manual instructions.

The isolation procedure utilizes 2 ml deep well plates with square shaped, round bottom format wells. The following deep well plates have been tested

and are recommended to be used with KingFisher 96: Greiner (n:o780270), Simport (n:o T110-10).

Sample preparation

The sample preparation protocol described here is designed for general use. Optimization for different proteins may be needed.

KingFisher 96 protocols are designed to use with *E. coli* bacterial pellets cultivated in 96 deep well plate. In general, 1 ml of LB medium with appropriate antibiotics is added to each well where single colonies are inoculated. The cultures are grown in incubator shaker o/n at 37°C, after which they are inoculated to fresh medium. Protein expression is induced (e.g. 1 mM IPTG) after OD₆₀₀ has reached 0.5-0.6. Cells are harvested after induction and the pellets can be stored at -20°C or -70°C. During cultivation the cultures may be protected against cross-contamination by covering the plate with porous tape sheet.

KingFisher 96 process

Fill the plates and start the process according to following instructions. For plate filling you can use pipetting station, Multidrop DW (Thermo Electron), multichannel pipette etc. See Ordering information for liquid handling products offered by Thermo Electron. Short pipetting instructions are given in table 2 followed by detailed instructions of plate filling and starting process.

Table 2. Short pipetting instruction for KingFisher 96 protocols

Plate type	96_His_tag_1		96_His_tag_2	
	Plate content	Plate name	Plate content	Plate name
KingFisher 96	KingFisher 96 tip comb for DW magnet	Tip plate	KingFisher 96 tip comb for DW magnets	Tip plate
Deep well	Bacterial pellet 600 µl Lysis buffer	Lysis	Bacterial pellet 600 µl Lysis buffer	Lysis
Deep well	40 µl Magnetic particles 900 µl TALON Binding and Washing Buffer	BeadWash	40 µl Magnetic particles 900 µl TALON Binding and Washing Buffer	BeadWash
Deep well	300 µl TALON Binding and Washing Buffer	Wash1	300 µl TALON Binding and Washing Buffer	Wash1
Deep well	300 µl TALON Binding and Washing Buffer	Wash2	300 µl TALON Binding and Washing Buffer	Wash2
KingFisher 96	100 µl of TALON Elution Buffer	Elution	100 µl TALON Binding and Washing Buffer	Release

1. Fill plates “Wash1” and “Wash2” (deep well plates) with 300 µl of TALON Binding and Washing Buffer.

2. Add 900 μ l of TALON Binding and Washing Buffer and 40 μ l magnetic particles into “BeadWash” plate (deep well plate).
3. In Protocol 96_His_tag_1, fill plate “Elution” (KingFisher 96 plate) with 100 μ l of TALON Elution Buffer
4. In Protocol 96_His_tag_2, fill plate “Release” (KingFisher 96 plate) with 100 μ l of TALON Binding and Washing Buffer.
5. Add 600 μ l of Lysis buffer to deep well plate containing bacterial pellet.
6. Combine the tip comb and KingFisher 96 plate (figure 1).
7. Select intended protocol using arrow keys.
8. Open the sliding door of the instrument if the lid is present.
9. Load the plates according to protocol request and confirm each action by pressing START.
 - Note! Be sure to place the plates 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.
10. After sound signal indicating the end of run remove plates according to protocol request. Confirm each action by pressing START.
11. To complete run press STOP.

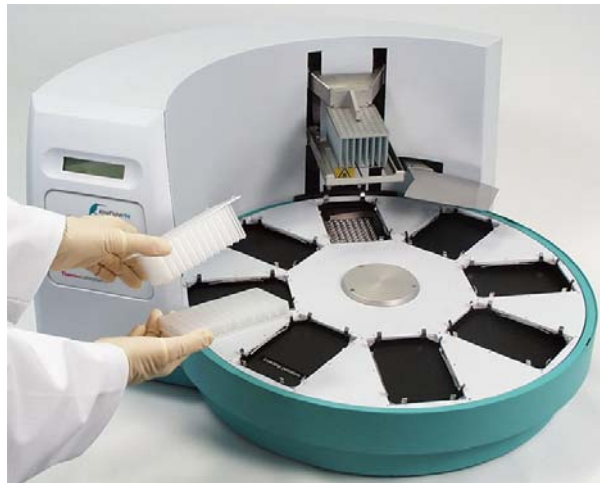


Figure 1. Combining the tip comb with KingFisher 96 plate.

KingFisher 96 protocol description

1. The bacterial pellet is lysed in deep well plate for 10 minutes.
2. Beads are prewashed and transferred into lysis plate.
3. The lysed sample is incubated with beads for 10 minutes.
4. Beads are washed twice in TALON Binding and Washing Buffer (Wash1 and Wash2 plates).
5. The purified protein is either eluted from beads into TALON Elution Buffer (protocol 96_His_tag_1) and beads are discarded into “Wash 1” plate.
6. **OR** The purified protein remains attached to beads (protocol 96_His_tag_2).

KingFisher mL

KingFisher mL protocols

Table 3. KingFisher mL protocols for His-tagged protein purification

Instrument	Protocol name	Protocol description
KingFisher mL	His_tag_mL_1	Used for purification of His-tagged proteins from <i>E. coli</i> lysates. Purified proteins are released from the magnetic particles into the TALON Elution Buffer.
KingFisher mL	His_tag_mL_2	Used for purification of His-tagged proteins from <i>E. coli</i> lysates. Magnetic particles with purified proteins attached are released to TALON Binding and Washing Buffer.

Sample preparation

The sample preparation protocol described here is designed for general use. Optimization for different proteins may be needed.

KingFisher mL protocols are designed to use with *E. coli* bacterial cultures grown and induced in shaking flasks or culture tubes. Inoculate culture flask/tube containing LB medium with appropriate antibiotics with o/n culture. Protein expression is induced (e.g. 1 mM IPTG) after OD₆₀₀ has reached 0.5-0.6. Cells are harvested after induction and the pellets can be stored at -20°C or -70°C.

Bacterial cell lysis is performed before setting the sample into the KingFisher mL instrument:

- Let the bacterial cell pellet melt on ice and add appropriate amount of lysis buffer (for 2 ml culture use 180 µl of lysis buffer). Lyse the cells 30 minutes on ice. Meanwhile add rest of the reagents to KingFisher mL tube strips according to instructions in table 4.
- After lysis transfer 160 µl of lysate into the tube 1 in KingFisher mL .

KingFisher mL process

Short pipetting instructions are given in table 4 followed by detailed instructions of plate filling and starting process.

Table 4. Short pipetting instructions for KingFisher mL protocols.

Tube no	His_tag_mL_1	His_tag_mL_2
	Tube content	Tube content
1	160 µl cell lysate 640 µl TALON Binding and Washing Buffer	160 µl cell lysate 640 µl TALON Binding and Washing Buffer
2	40 µl magnetic particles 900 µl TALON Binding and Washing Buffer	40 µl magnetic particles 900 µl TALON Binding and Washing Buffer
3	1000 µl TALON Binding and Washing Buffer	1000 µl TALON Binding and Washing Buffer
4	1000 µl TALON Binding and Washing Buffer	1000 µl TALON Binding and Washing Buffer
5	100 µl TALON Elution Buffer	100 µl TALON Binding and Washing Buffer

1. Place an appropriate number of tube strips needed for the samples (one tube strip per sample) into removable tube strip tray.
2. Add 1000 µl TALON Binding and Washing Buffer into tubes 3 and 4.
3. In protocol His_tag_mL_1, add 100 µl of TALON Elution Buffer into tube 5.
4. In protocol His_tag_mL_2, add 100 µl of TALON Binding and Washing Buffer into tube 5.
5. Add 40 µl of magnetic particles and 900 µl of TALON Binding and Washing Buffer into tube 2.
6. Add 160 µl of cell lysate and 640 µl of TALON Binding and Washing Buffer into tube 1.
7. Insert the tube strip tray to the instrument and insert the tips combs into the slots.
8. Close the front lid and start the process by selecting intended protocol using arrow keys and by pressing START.
9. Remove the tube-strip tray from the KingFisher mL after program has completed.

KingFisher mL protocol description

1. Magnetic particles are washed and transferred to tube 1.
2. The cell lysate is incubated with particles in tube 1 for 10 minutes.
3. The particles are washed twice in tubes 3 and 4.
4. The purified protein is either eluted from beads into TALON Elution Buffer (protocol His_tag_mL_1) and beads are discarded into tube 3.
5. **OR** The purified protein remains attached to beads (protocol His_tag_mL_2).

Trouble shooting

1. Magnetic particles are not collected efficiently.
 - Use of Tween-20 (~0.01%) in reagents will enhance collection.
2. Extra washing steps needed for the sample.
 - Modify the KingFisher protocol using KingFisher Software.
3. Longer incubation time needed.
 - Modify the KingFisher protocol using KingFisher Software.
4. Who should I contact if I have problems with KingFisher instrument?
 - Contact your local Thermo Electron representative

Ordering information

KingFisher instruments and consumables

Product number	Product description
540 00 50	KingFisher mL magnetic particle processor
540 05 00	KingFisher 96 magnetic particle processor
970 02 131	KingFisher mL Combi 60 (tubes and tip combs for 60 samples)
970 02 530	KingFisher 96 tip comb for DW magnets, 10 pcs / box
970 02 540	KingFisher 96 plate (200 µl), 48 plates / box

Related products for liquid handling

Product number	Product description
454 00 00	Finnpipette Stepper
4610050	Finnpipette Focus Multichannel Pipette, 30-300 µl
584 01 70	Multidrop DW, 220-240 V 50/60 Hz
584 01 77	Multidrop DW, 100-120 V 50/60 Hz

Contact information

Thermo Electron Corporation
Ratastie 2, P.O. Box 100
FIN-01621 Vantaa
Finland
Tel. +358-9-329100
Fax. +358-9-32910415
www.thermo.com