Instruction Manual

Thermo Scientific KingFisher Pure Plasmid Kit

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NOTE: For more details on storing the kit reagents, refer to "Storage Conditions" on page 6.

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Kit Content

Table 1-1. Thermo Scientific™ KingFisher™ Pure Plasmid Kit

Item	KingFisher Pure Plasmid Kit	
Cat. No.	98080196	98080496
Package size	96 samples	384 samples
RNase A	0.28 ml	1 ml
Resuspension Solution	25 ml	90 ml
Lysis Buffer	25 ml	90 ml
Neutralization Solution	25 ml	90 ml
KingFisher Magnetic Beads	2 x 1.4 ml	10.6 ml
Wash Buffer 1 (conc.)*	110 ml	3 x 110 ml
Wash Buffer 2 (conc.)*	60 ml	3 x 60 ml
Elution Buffer	18 ml	45 ml

* Addition of ethanol and/or isopropanol required.

The KingFisher Pure Plasmid Kit (Cat. No. 98080196 or 98080496) is intended for the purification of plasmid DNA, using the Thermo Scientific™ KingFisher™ Flex with a 96 deep well head or the Thermo Scientific™ KingFisher™ Duo with a 12-pin head from an overnight *E. coli* culture.

The user will need the KingFisher Flex or KingFisher Duo magnetic particle processor for conducting purification (Table 1-2). In addition, several common laboratory instruments and consumables are necessary to conduct an efficient purification. For more details, refer to Chapter 5: "Protocols and Pipetting Instructions". Suitable consumables for the KingFisher Duo and KingFisher Flex are listed in Table 1-3 and Table 1-4.

Storage Conditions

Upon arrival of the kit, store the Thermo Scientific™ KingFisher™ Magnetic Beads at +4°C. The RNase A solution is stable at room temperature as long as the vial remains sealed. After being opened, it should be stored at -20°C. After the addition of RNase A, the Resuspension Solution is stable for six months when stored at +4°C. Other components of the kit should be stored at room temperature (15–25°C). The reagents are stable for up to three years from the manufacturing date.

Additional Reagents Required

- 96–100% ethanol (EtOH), molecular biology grade
- 100% isopropanol, molecular biology grade

Table 1-2. Thermo Scientific™ KingFisher™ magnetic particle processors

Cat. No.	Product
5400110	KingFisher Duo Prime magnetic particle processor
5400630	KingFisher Flex magnetic particle processor with 96 deep well head

Table 1-3. Thermo Scientific™ KingFisher™ Flex consumables

Cat. No.	Product	Package size
97002534	KingFisher Flex 96 tip comb for deep well magnet	100 pcs
97002540	KingFisher Flex 96 KF plate (200 µl)	48 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs

Table 1-4. Thermo Scientific™ KingFisher™ Duo consumables

Cat. No.	Product	Package size
97003500	KingFisher Duo 12-tip comb for Microtiter deep well 96 plate	50 pcs
97003520	KingFisher Duo elution strip	40 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate (tips combs, plates, and elution strips for 96 samples)	1 box

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Product Description

Introduction

The KingFisher Pure Plasmid Kit is designed for rapid automated purification of high-copy number plasmids from an overnight *E. coli* culture using Thermo Scientific™ KingFisher™ instruments. The plasmid DNA purified using the KingFisher Pure Plasmid Kit is of high quality and free of proteins, genomic DNA, nucleases, and other contaminants or inhibitors. It is, therefore, suitable for direct use in many different downstream applications, such as transformation of bacteria, PCR (polymerase chain reaction), restriction endonuclease digestion, automated sequencing, in vitro transcription, and other enzymatic reactions.

Intended Use

The KingFisher Pure Plasmid Kit is developed for purification of plasmid DNA from cultured *E. coli* using paramagnetic particles. The reagents and specific plastic consumables are designed for use with the KingFisher Flex and KingFisher Duo magnetic particle processors as part of an integrated system. The KingFisher Pure Plasmid Kit is only intended for research use, not for clinical or diagnostic use. The user is responsible for validating the performance of the KingFisher instrument and the KingFisher Pure Plasmid Kit for any particular use.

Principle and Procedure

The KingFisher Pure Plasmid Kit uses magnetic-particle technology for DNA purification. The Thermo Scientific™ KingFisher™ technology combines the speed and efficiency of DNA purification with easy handling of magnetic particles. The purification process requires no phenol/chloroform extraction and needs very little hands-on time.

Pelleted bacterial cells are resuspended and subjected to SDS/alkaline lysis to liberate plasmid DNA. The lysate is neutralized allowing denatured plasmid DNA to reanneal. Meanwhile, cell debris, such as proteins, chromosomal DNA, and SDS are precipitated and can be pelleted by centrifugation. Next, plasmid DNA binds to the surface of the KingFisher Magnetic Beads and impurities are effectively removed during the subsequent wash steps. High-quality plasmid DNA is eluted into the Elution Buffer, and is ready for subsequent downstream processes.

Kit Specifications

The KingFisher Pure Plasmid Kit is designed for rapid automated preparation of highly pure plasmid DNA from *E. coli* strains using KingFisher magnetic particle processors.

Fresh or frozen overnight *E. coli* culture can be used.

High-quality plasmid DNA can be obtained from various *E. coli* strains, including DH10B, DH5α, JM109, JM107, or TOP10. Common plasmid vectors of various length and copy number can be efficiently purified by the kit.

The procedure is optimized for use with bacterial cultures grown in Luria-Bertani (LB) media. The use of rich growth media may give higher yields.

The approximate processing time is 40 minutes for the purification of 96 samples on the KingFisher Flex and 12 samples on the KingFisher Duo. The obtained DNA can be used directly in various downstream applications.

Typically 5–12 µg of plasmid DNA can be purified from 1 ml of overnight bacterial culture with high-copy number plasmid, with an A_{260}/A_{280} ratio of ≥ 1.7–2.0.

The yields of acquired purified DNA depend on the bacterial strain, plasmid copy number, and the method of culturing.

KingFisher Magnetic Particle Processors

The KingFisher magnetic particle processors are designed for the automated transfer and processing of magnetic particles in microplate format. The patented technology of the Thermo Scientific™ KingFisher™ systems is based on the use of magnetic rods covered with a disposable, specially designed tip comb and plates or tubes. Use only Thermo Scientific™ KingFisher™ plastic consumables, as use of products from other

manufacturers may cause unsuitable mixing or even instability in the KingFisher instrument. The instrument functions without any dispensing or aspiration parts or devices. Samples and reagents, including magnetic particles, are dispensed onto the plates according to the corresponding instructions. Dispensing can be carried out manually or partially automatically using automatic dispensers, for example, the Thermo Scientific™ Multidrop™ Combi and/or the Thermo Scientific™ Versette™. Thermo Scientific™ BindIt™ Software 3.2 (or newer version of software) can be used for running ready-made and optimized protocols for the Thermo Scientific™ KingFisher™ Pure Kits. It is also possible to transfer the developed protocol onto the onboard software and run it directly from the instrument. The KingFisher instruments provide a rapid and automated solution for complicated and time-consuming purification processes, resulting in high-purity DNA without risk of carryover or cross-contamination.

The KingFisher instrument family comprises four systems covering working volumes from 20 to 5000 µl. Each system consists of an instrument, specially designed plastic consumables, and the easy-to-use BindIt Software. The KingFisher Pure Plasmid Kit is optimized and ready for use with the KingFisher Flex or KingFisher Duo.

KingFisher magnetic particle processors are intended for professional research use by trained personnel. Detailed information and user instructions for the KingFisher instruments can be found in their respective user manuals.

The BindIt Software protocols optimized for the KingFisher Pure Plasmid Kit are available for the KingFisher Flex 96 and 24 and KingFisher Duo. For more information, go to www.thermoscientific.com/kingfisherinfo or contact your local authorized distributor.

Table 2-1. Overview of KingFisher Flex and KingFisher Duo magnetic particle processors

	KingFisher Flex		KingFisher Duo	
	96 deep well formats	24 format	12 format	6 format
Processing volume	20–1000 µl*	200–5000 µl	30–1000 µl*	200–5000 µl
Capacity	Up to 96 samples per run (sample volume approx. 200 µl)	Up to 24 samples per run (sample volume approx. 1 ml)	Up to 12 samples per run (sample volume approx. 200 µl)	Up to 6 samples per run (sample volume approx. 1 ml)
Magnetic head	96 inter-changeable formats for Microtiter deep well 96 plate, PCR plate and KingFisher Flex 96 KF plate	24 format for KingFisher Flex 24 deep well plate	12-pin magnet head for Microtiter deep well 96 plate	6-pin magnet head for KingFisher Flex 24 deep well plate
Plates	KingFisher Flex 96 KF plate (20–200 µl), 96 well PCR plate, skirted (20–100 µl), Microtiter deep well 96 plate (50–1000 µl)	KingFisher Flex 24 deep well plate (200–5000 µl)	Microtiter deep well 96 plate (50–1000 µl), KingFisher Duo elution strip (30–130 µl)	KingFisher Flex 24 deep well plate (200–5000 µl)
Tip combs	KingFisher Flex 96 tip comb for PCR magnets, KingFisher Flex tip comb for KF magnets, KingFisher Flex 96 tip comb for deep well magnets	KingFisher Flex 24 tip comb for deep well magnets	KingFisher Duo 12-tip comb	KingFisher Duo 6-tip comb
Heating temperature	Heating block temperature from +5°C above ambient room temperature to +115°C		Heating block temperature from +10°C to +75°C, elution strip +4°C to +75°C at room temperature	

* See the details above on the Plates row.

3

Safety Information

Always wear a laboratory coat, disposable gloves and goggles, and follow the safety instructions provided in the kit instruction manual. It is recommended that Good Laboratory Practice (GLP) is followed to guarantee reliable analyses.

The following components of the KingFisher Pure Plasmid Kit contain hazardous contents.

Lysis Buffer

Signal word Danger
Hazard content Sodium hydroxide 0,1-1 %
Hazard statement
H314 Causes severe skin burns and eye damage.
Precautionary statements
P260 Do not breathe dust/fume/gas/mist/vapours/spray.
P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/physician.
P405 Store locked up.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Wash Buffer 1 (concentrated)

Signal word Warning
Hazard content Guanidium chloride 25-50 %
Hazard statements
H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
Precautionary statements
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P321 Specific treatment (see on this label).
P362 Take off contaminated clothing and wash before reuse.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

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Storage Conditions and Preparation of the Reagents

Storage Conditions

Upon arrival of the kit, store the KingFisher Magnetic Beads at +4°C. The RNase A solution is stable at room temperature as long as the vial remains sealed. After being opened, it should be stored at -20°C. After the addition of RNase A, the Resuspension Solution is stable for six months when stored at +4°C. Other components of the kit should be stored at room temperature (15–25°C). The reagents are stable for up to three years from the manufacturing date.

Preparation of the Resuspension Solution

Add the RNase A solution included in the kit to the Resuspension Solution and mix thoroughly. After the addition of RNase A, the Resuspension Solution is stable for six months when stored at +4°C.

For longer storage, aliquot the Resuspension Solution into an appropriate number of aliquots and supplement one aliquot with 10 µl of RNase A per 1 ml of Resuspension Solution. Store the remaining RNase A at -20°C.

Preparation of the Wash Buffers

Add isopropanol and 96–100% ethanol to concentrated Wash Buffer 1 and Wash Buffer 2, as indicated below in Table 4-1 prior to the first use.

Table 4-1. Instructions for the preparation of the buffers. Add the indicated volume per bottle.

	96 samples (Cat. No. 98080196)		**384 samples (Cat. No. 98080496)**	
	Wash Buffer 1	Wash Buffer 2	Wash Buffer 1	Wash Buffer 2
Concentrated buffer	110 ml	60 ml	110 ml	60 ml
Isopropanol	55 ml	–	55 ml	–
Ethanol (96–100%)	55 ml	162 ml	55 ml	162 ml
Total volume	220 ml	222 ml	220 ml	222 ml

After preparing each solution, mark the bottle to indicate that the step has been completed. The buffers can be stored at room temperature.

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Protocols and Pipetting Instructions

Before beginning the DNA purification protocol, carefully read through the *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. No. N07669) or the *Thermo Scientific™ KingFisher™ Duo User Manual* (Cat. No. N12420), and the *Thermo Scientific™ BindIt™ Software for KingFisher Instruments User Manual* (Cat. No. N07974).

BindIt Software protocols for the KingFisher Pure Plasmid Kit can be found in BindIt Software or at www.thermoscientific.com/kingfisher.

Handling of KingFisher Magnetic Beads

A homogeneous distribution of the KingFisher Magnetic Beads in the container is essential before the beads are transferred to the wells in order to ensure a high consistency between the wells. To gain complete resuspension of the beads, shake the container vigorously or vortex briefly.

Instructions for KingFisher Flex with 96 Deep Well Plates

These instructions are intended for plasmid DNA purification from bacterial cells pelleted from 0.5–5 ml of overnight *E. coli* cultures, using the KingFisher Pure Plasmid Kit (Cat. No. 98080196 or 98080496) and the KingFisher Flex with 96 deep well plates. An OD_{600} of 2.0–6.0 for cultures with high-copy number plasmids ensures that bacteria have reached the proper growth density for harvesting and plasmid DNA isolation. Using cultures that have OD_{600} readings > 6.0 may lead to incomplete processing of the bacterial lysate and decreased purity of isolated plasmid DNA.

When using the KingFisher Pure Plasmid Kit for the first time, add the RNase A solution included in the kit to the Resuspension Solution. Then prepare the Wash Buffer 1 and Wash Buffer 2. For detailed instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Reagents".

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C). Do not shake the Lysis Buffer vigorously.

1. Resuspend pelleted bacterial cells in 200 µl of Resuspension Solution containing RNase A. The bacterial pellet should be completely resuspended by shaking or pipetting up and down until no cell clumps remain.

2. Add 200 µl of Lysis Solution and mix gently by shaking the samples 4–6 times until the solution becomes viscous and slightly clear. Incubate for 2 min at room temperature.

NOTE: Do not shake intensively to avoid shearing chromosomal DNA. Do not incubate for more than 2 min to avoid denaturation of supercoiled plasmid DNA.

3. Add 200 µl of Neutralization Solution and mix immediately by shaking the samples 4–6 times.

4. Add 50 µl of isopropanol (100%) and mix immediately by shaking the samples 4–6 times.

5. To clear the lysate, centrifuge the samples for 10 min at 3,800–4,000 x g to pellet the cell debris and the chromosomal DNA.

6. Prepare the Sample plate (i.e. a Thermo Scientific™ Microtiter™ deep well 96 plate) as follows. Add 25 µl of magnetic bead suspension and 250 µl of isopropanol to each well. Slowly aspirate 500 µl of the clear lysates and transfer them to the Sample plate. Pipet carefully from the top of the supernatant to avoid touching the pelleted flocculent debris. Mix the content of the Sample plate immediately by shaking the plate 4–6 times. Leave the plate at room temperature while the other plates are being filled.

Add the following reagents to the Sample plate.

Plate number	Plate type	Plate name	Content	Reagent volume
1	Microtiter deep well 96 plate	Sample	Bacterial cell lysate	500 µl
			Isopropanol	250 µl
			KingFisher Magnetic Beads*	25 µl

* Resuspend the KingFisher Magnetic Beads well by vortexing before use.

7. Take four empty Microtiter deep well 96 plates and an empty Thermo Scientific™ KingFisher™ Flex 96 KF plate and fill them as follows.

Plate number	Plate type	Plate name	Content	Reagent volume
2	Microtiter deep well 96 plate	Wash 1_1	Wash Buffer 1	800 µl
3		Wash 1_2	Wash Buffer 1	700 µl
4		Wash 2_1	Wash Buffer 2	700 µl
5		Wash 2_2	Wash Buffer 2	700 µl
6	KingFisher Flex 96 KF plate	Elution	Elution Buffer	100 µl

8. Place a Thermo Scientific™ KingFisher™ Flex 96 tip comb for deep well magnets on a Tip Plate (i.e. an empty KingFisher Flex 96 KF plate).

9. Start the PURE_Plasmid_Flex96 protocol using the KingFisher Flex 96 and load the plates.

Switch on the KingFisher Flex making sure that you are using the Thermo Scientific™ KingFisher™ Flex 96 deep well head and heating block. Connect the PC with BindIt Software to the KingFisher Flex. Start the PURE_Plasmid_Flex96 protocol. Insert the Tip Plate and the filled plates into the instrument as instructed on the KingFisher Flex display. After all the plates have been loaded into the instrument, the protocol will start.

When the KingFisher Flex is to be run as a standalone instrument, transfer the PURE_Plasmid_Flex96 protocol to the KingFisher Flex. The instructions for transferring the protocol can be found in Chapter 4: “Using the software” in the *BindIt Software for KingFisher Instruments User Manual*.

10. After the run is completed, remove the plates and store the purified DNA.

When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and switch off the instrument. Store the purified plasmid DNA accordingly. The purified DNA is ready for use in downstream applications.

NOTE: The final DNA concentration in the Elution Buffer may increase if the purified plasmid DNA is eluted into a smaller than recommended volume of buffer, but this can slightly reduce the overall DNA yield.

Instructions for KingFisher Duo with 12-pin Magnet Head

These instructions are intended for DNA purification from bacterial cells pelleted from 0.5–5 ml of overnight *E. coli* cultures, using the KingFisher Pure Plasmid Kit (Cat. No. 98080196 or 98080496) and the KingFisher Duo with 12-pin magnet head.

When using the KingFisher Pure Plasmid Kit for the first time, add the RNase A solution included in the kit to the Resuspension Solution. Then prepare the Wash Buffer 1 and Wash Buffer 2. For detailed instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Reagents".

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C). Do not shake the Lysis Buffer vigorously.

1. Resuspend pelleted bacterial cells in 200 µl of Resuspension Solution containing RNase A. The bacterial pellet should be completely resuspended by shaking or pipetting up and down until no cell clumps remain.

2. Add 200 µl of Lysis Solution and mix gently by shaking the samples 4–6 times until the solution becomes viscous and slightly clear. Incubate for 2 min at room temperature.

NOTE: Do not shake intensively to avoid shearing chromosomal DNA. Do not incubate for more than 2 min to avoid denaturation of supercoiled plasmid DNA.

3. Add 200 µl of Neutralization Solution and mix immediately by shaking the samples 4–6 times.

4. Add 50 µl of isopropanol (100%) and mix immediately by shaking the samples 4–6 times.

5. To clear the lysate, centrifuge the samples for 10 min at 3,800–4,000 x g to pellet the cell debris and the chromosomal DNA.

6. Take one empty Microtiter deep well 96 plate and one Thermo Scientific™ KingFisher™ Duo elution strip.

7. Prepare the Plasmid DNA plate (i.e. a Microtiter deep well 96 plate).

Add the following reagents to the rows (see the table below). Note that row B is reserved for the tip comb and should be left *empty*. Note that rows C and D are also left *empty*. Resuspend the KingFisher Magnetic Beads well (e.g. by vortexing) before removing them from the bottle.

Slowly aspirate 500 µl of the clear lysates and transfer them to row A. Pipet carefully from the top of the supernatant to avoid touching the pelleted flocculent debris. Mix the content of the Sample plate immediately by shaking the plate 4–6 times.

Plate name and type	Row	Row name	Content	Reagent / Sample volume per well
Plasmid DNA plate Microtiter deep well 96 plate	A	Bacterial lysates	Bacterial cell lysate Isopropanol KingFisher Magnetic Beads*	500 µl 250 µl 25 µl
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Wash 1_1	Wash Buffer 1	800 µl
	F	Wash 1_2	Wash Buffer 1	700 µl
	G	Wash 2_1	Wash Buffer 2	700 µl
	H	Wash 2_2	Wash Buffer 2	700 µl

* Resuspend the KingFisher Magnetic Beads by vortexing thoroughly before use.

8. Fill the KingFisher Duo elution strip as follows. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and the Elution Buffer is pipetted into the correct wells.

Elution strip	Content	Reagent volume per well
KingFisher Duo elution strip	Elution Buffer	100 µl

9. Place a Thermo Scientific™ KingFisher™ Duo 12-tip comb into row B on the Plasmid DNA plate.

9. Start the PURE_Plasmid_Duo protocol using the KingFisher Duo and load the plate and elution strip.

Switch on the KingFisher Duo making sure that you are using the Thermo Scientific™ KingFisher™ Duo 12-pin magnet head and heating block. Connect the PC with BindIt Software to the KingFisher Duo. Start the PURE_Plasmid_Duo protocol. Insert the Plasmid DNA plate and elution strip into the instrument as indicated on the KingFisher Duo display and press **OK**. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user.

When the KingFisher Duo is to be run as a standalone instrument, transfer the PURE_Plasmid_Duo protocol to the KingFisher Duo. The instructions for transferring the protocol can be found in Chapter 4: “Using the software” in the *BindIt Software for KingFisher Instruments User Manual*.

7. After the run is completed, remove the plate and elution strip and store the purified DNA.

When the protocol is completed, remove the plate and elution strip according to the instructions on the KingFisher Duo display and switch off the instrument. Store the purified plasmid DNA accordingly. The purified DNA is ready for use in downstream applications.

NOTE: The final DNA concentration in the Elution Buffer may increase if the purified plasmid DNA is eluted into a smaller than recommended volume of buffer, but this can slightly reduce the overall DNA yield.

Quantification and Determination of the Purity of DNA

It is recommended to measure the absorbance at 320 nm, 280 nm, and 260 nm. The concentration of DNA can be defined with the absorbance at 260 nm (A_{260}). One unit at 260 nm corresponds to 50 µg of DNA per ml. The ratio between the A_{260}/A_{280} indicates the purity of the DNA. The value for DNA should be ≥ 1.7–2.0.

It is recommended that A_{320} correction is used for the absorbance values. Subtract the A_{320} from the A_{260} and A_{280} ratios to remove the effects of carryover of the magnetic particles.

- Concentration of DNA sample = 50 µg/ml x ($A_{260} - A_{320}$) x dilution factor
- Total amount of DNA isolated = concentration x volume of sample in ml
- Purity of DNA sample = ($A_{260} - A_{320}$)/($A_{280} - A_{320}$)

6

General Information

Reagent Specificity and Volumes

A reagent must not be used with any kit other than that for which it is intended. It is strongly recommended that the volume of reagents in each well or tube is kept within the limits specified in the *KingFisher Flex User Manual* or *KingFisher Duo User Manual* to avoid spillover and to maximize efficiency of performance.

Handling of Magnetic Beads

The KingFisher Magnetic Beads should be mixed thoroughly before use to avoid the risk of transferring variable amounts of the beads to the respective wells or tubes. The amount of beads in the wells or tubes affects the yield of the purified DNA.

Binding, Wash, and Elution Steps

The binding between the purified DNA and the KingFisher Magnetic Beads is strong in the presence of a chaotropic salt. The binding will remain throughout the wash steps until the elution where the DNA is released.

The volume of Elution Buffer can be modified depending on user requirements concerning the purified DNA concentration. The final DNA concentration may increase if the purified DNA is eluted into a smaller than recommended volume of Elution Buffer, but this can slightly reduce the overall DNA yield. The modifications of the elution step must be done in BindIt Software and according to the volume ranges suitable for the KingFisher instrument. The table below indicates the available elution volumes of the KingFisher instruments.

Table 6-1. Available elution volumes of the KingFisher Flex and KingFisher Duo

KingFisher instrument	Elution volumes
KingFisher Flex with 96 deep well head, elution in a KingFisher Flex 96 KF plate	50–150 µl
KingFisher Flex with 96 deep well head, elution in a Microtiter deep well 96 plate	50–1000 µl
KingFisher Flex with 24 deep well head	200–5000 µl
KingFisher Duo with 12-pin magnet head, elution in an elution strip	30–130 µl
KingFisher Duo with 12-pin magnet head, elution in a Microtiter deep well 96 plate	50–1000 µl

To maximize the yield of purified DNA, avoid the lowest permitted elution volumes in the KingFisher instruments. The Elution Buffer should cover the KingFisher Magnetic Beads completely, and any possible magnetic-bead pellet(s) should be completely resuspended. In addition, the volume of Elution Buffer should be adequate for efficient mixing of the beads in order to obtain a maximal release of the purified DNA from the beads.

Decontamination and Disinfection of Sample Material

You should decontaminate the sample material and the reagents and plastics that have been in contact with the sample material in order to minimize the risk of contamination. Use a decontaminant, such as Virkon™, paying due attention to the manufacturer's instructions. You should also take care of the appropriate treatment and/or disposal of waste.

A

Troubleshooting

Problem	Possible cause and actions
	Old bacterial culture. Prepare new starter culture by inoculating a freshly-isolated single bacterial colony in antibiotic-containing growth medium and grow bacteria according to standard protocols. **Incomplete bacterial cell lysis.** It is essential that the cell pellet is completely resuspended in Resuspension Solution prior to lysis. There should be no visible cell clumps before adding the Lysis Solution. Check the Lysis Solution for salt precipitation before each use. Redissolve any precipitate by warming the solution to 37°C, then mix well and cool to 25°C before use. Use overnight culture with an OD_{600} = 2–6. After centrifugation, avoid transferring pelleted cell debris to a new tube or deep well plate (prefilled with 25 µl of magnetic bead suspension and 250 µl of isopropanol).

Continued

Cont.

Problem	Possible cause and actions
Low DNA yield	**Isopropanol and / or ethanol were not added to Wash Buffer 1.** Ensure that isopropanol and ethanol were added to Wash Buffer 1 before the first use. Follow the instructions to prepare Wash Buffer 1 on page 16. **Ethanol was not added to Wash Buffer 2.** Ensure that ethanol was added to Wash Buffer 2 before the first use. Follow the instructions to prepare Wash Buffer 2 on page 16. There should be an adequate volume of Elution Buffer to completely cover the KingFisher Magnetic Beads during the elution step. Do not let the KingFisher Magnetic Beads dry as this may result in lower elution efficiency. Use only Thermo Scientific plates, strips, and tip combs with the KingFisher instruments. Use of products from other manufacturers may cause unsuitable mixing and affect the yield of purified DNA.
Low purity	**Prolonged storage** of the sample material may reduce the quality and quantity of the plasmid DNA. **Insufficient washing** causes impurities in the eluted DNA. Residual salt remaining in the plasmid preparation may inhibit downstream enzymatic reactions. Use the correct order for the Wash Buffers. Follow the instructions to prepare the Wash Buffers on page 16.
RNA contamination	**RNase A was not added to the Resuspension Solution.** Ensure that the RNase A was added to the Resuspension Solution, as described on page 15.
Genomic DNA contamination	**Samples vigorously vortexed or shook during cell lysis or neutralization steps.** To avoid genomic DNA contamination, mix the solution by gently inverting the tube or by shaking the plate 5–8 times during the lysis and neutralization steps. Do not allow the cell lysis step to proceed for more than 2 min. Do not cultivate cells longer than 16 h in LB media. Ensure that isopropanol was added before centrifugation. Transfer lysed sample carefully from the top of the supernatant to avoid touching the pelleted flocculent. Residual genomic DNA can be removed from purified plasmid DNA by treatment using T7 DNA Polymerase.

Continued

Cont.

Problem	Possible cause and actions
Purified sample contains additional plasmid forms	**Plasmid DNA denatured during cell lysis.** Denatured plasmid DNA migrates ahead of supercoiled DNA and is not suitable for enzymatic manipulations, such as restriction digestion. To avoid denaturation, do not allow the cell lysis to proceed for more than 2 min.
Magnetic particles remaining in the sample or elution well	Starting material that is too viscose prevents efficient collection of the KingFisher Magnetic Beads from the lysed sample. The magnetic rods will not be able to collect all the particles unless the viscose samples are diluted before the beginning of the purification process. Improper lysis may also cause problems collecting the KingFisher Magnetic Beads. If the KingFisher Magnetic Beads are inefficiently collected from the elution step, the addition of a small amount of detergent (e.g. Tween™ 20) may improve the results. KingFisher Magnetic Beads that occasionally remain attached to the tip combs at the end of the process do not affect the plasmid DNA yield, as the DNA has already been released from the KingFisher Magnetic Beads into the Elution Buffer. If the KingFisher magnetic particle processor does not work properly, refer to the relevant user manual of the KingFisher instrument in use.

B

Ordering Information

Table B-1. KingFisher Pure Plasmid Kits

Cat. No.	Product	Package size
98080196	KingFisher Pure Plasmid Kit	96
98080496	KingFisher Pure Plasmid Kit	384

Table B-2. KingFisher Flex consumables

Cat. No.	Product	Package size
97002514	KingFisher Flex 96 tip comb for PCR magnet	80 pcs
97002524	KingFisher Flex 96 tip comb for KF magnet	100 pcs
97002534	KingFisher Flex 96 tip comb for deep well magnet	100 pcs
97002610	KingFisher Flex 24 deep well tip comb and plate	50 pcs
97002540	KingFisher Flex 96 KF plate (200 µl)	48 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
95040470	KingFisher Flex 24 deep well plate	50 pcs
95040480	KingFisher Flex 24 deep well plate, sterile	50 pcs

Table B-3. KingFisher Duo consumables

Cat. No.	Product	Package size
97003500	KingFisher Duo 12-tip comb for Microtiter deep well 96 plate	50 pcs
97003510	KingFisher Duo 6-tip comb for KingFisher Flex 24 deep well plate	48 pcs
97003520	KingFisher Duo elution strip	40 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
95040470	KingFisher Flex 24 deep well plate	50 pcs
95040480	KingFisher Flex 24 deep well plate, sterile	50 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate (tips combs, plates, and elution strips for 96 samples)	1 box

Notes

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