Instruction Manual

Thermo Scientific KingFisher Pure DNA Plant Kit

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Table of Contents

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

1

Kit Content

Table 1-1. Thermo Scientific™ KingFisher™ Pure DNA Plant Kit

Item	KingFisher Pure DNA Plant Kit	
Cat. No.	98050196	98050496
Package size	96 samples	384 samples
Lysis Buffer A	60 ml	225 ml
Lysis Buffer B	8.4 ml	32 ml
Precipitation Solution	16.5 ml	60 ml
KingFisher Magnetic Beads	2 x 1.4 ml	10.6 ml
RNase A	2 x 1.4 ml	7 x 1.2 ml
Wash Buffer 1 (conc.)*	50 ml	2 x 50 ml
Wash Buffer 2 (conc.)*	60 ml	3 x 60 ml
Elution Buffer	30 ml	70 ml

* Addition of ethanol required.

The KingFisher Pure DNA Plant Kit (Cat. No. 98050196 or 98050496) is intended for the purification of genomic DNA from plant material, using the Thermo Scientific™ KingFisher™ Flex with a 96 deep well head or the Thermo Scientific™ KingFisher™ Duo with a 12-pin head and a sample amount of up to 50 mg.

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, store the Thermo Scientific™ KingFisher™ Magnetic Beads at +4°C. All the other buffers and reagents included in the KingFisher Pure DNA Plant Kit can be stored at room temperature (15–25°C). The RNase A solution is stable at room temperature until the seal of the vial is broken. After being opened, the vial should be stored at -20°C. The reagents are stable for up to three years from the manufacturing date.

Additional Reagents Required

- 96–100% ethanol (EtOH), molecular biology grade
- Dithiothreitol (DTT) for seeds
- Polyvinylpyrrolidone (PVP) for woody, lignified, or polyphenol-rich samples

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

Cat. No.	Product
5400100	KingFisher Duo 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

2

Product Description

Introduction

The KingFisher Pure DNA Plant Kit is designed for rapid automated purification of DNA from ≤ 50 mg of plant material using Thermo Scientific™ KingFisher™ instruments. The DNA purified using the KingFisher Pure DNA Plant Kit is of high quality and free of proteins, nucleases, and other contaminants or inhibitors. It is, therefore, suitable for direct use in many different downstream applications, such as PCR (polymerase chain reaction), restriction analysis, and several other enzymatic reactions.

Intended Use

The KingFisher Pure DNA Plant Kit is developed for purification of DNA from plant material 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 DNA Plant Kit is suitable for extraction of DNA from fresh or frozen plant material. The KingFisher Pure DNA Plant Kit is only intended for research use, not for clinical or diagnostic use. The user is responsible for validating the performance of the Thermo Scientific™ KingFisher™ instrument and the KingFisher Pure DNA Plant Kit for any particular use, as the performance of the kits has not been validated for any specific species or downstream application.

Principle and Procedure

The KingFisher Pure DNA Plant 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.

The first step of the protocol binds the DNA from the homogenized plant material to the surface of the KingFisher Magnetic Beads in the presence of the Binding Buffer. The KingFisher Magnetic Beads are highly reactive, superparamagnetic beads. The following three effective wash steps dispose of proteins, cell debris, and any residual contaminants, while the DNA is bound to the KingFisher Magnetic Beads and transferred through the wash steps. Two different Wash Buffers are used, followed by an air drying step, which improves the purity of the DNA. High-quality DNA is eluted into the Elution Buffer, and is ready for subsequent downstream processes.

Kit Specifications

The KingFisher Pure DNA Plant Kit is designed for rapid automated preparation of highly pure DNA from plant material using KingFisher magnetic particle processors. If the homogenization of the starting material is excluded, the approximate processing time is 40 minutes for the purification of 96 samples on the KingFisher Flex and 12 samples on the KingFisher Duo when the sample volume is 400 µl. The obtained DNA can be used directly in various downstream applications.

Fresh or frozen plant material equaling ≤ 50 mg can be used. The procedure is optimized for sample volumes of 400 µl. Depending on the sample, up to 15 µg of DNA can be purified from 50 mg of fresh plant material with an A_{260}/A_{280} ratio of ≥ 1.7–2.0. The DNA yields depend on the sample type, sample storage, and homogenization method.

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 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 the 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 3.2. The KingFisher Pure DNA Plant 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 3.2 protocols optimized for the KingFisher Pure DNA Plant Kit are available for the KingFisher Flex 96 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 formats	24 format	12 format	6 format
Processing volume	20–1000 µl*	200–5000 µl	50–1000 µl*	200–5000 µl
Capacity	Up to 96 samples per run (sample volume 200 µl)	Up to 24 samples per run (sample volume 1 ml)	Up to 12 samples per run (sample volume 200 µl)	Up to 6 samples per run (sample volume 1 ml)
Magnetic head	96 inter-changeable formats for PCR plate, KingFisher Flex 96 KF plate, Microtiter deep well 96 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

The following components of the KingFisher Pure DNA Plant Kit contain hazardous contents (Table 3-1).

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.

Table 3-1. Safety precautions

Reagent	Hazardous contents	Safety instructions
Wash Buffer 1 (conc.)	Guanidinium chloride	Harmful if swallowed. Irritating to eyes and skin. Do not breathe gas/fumes/ vapor/spray. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves. This material and its container must be disposed of as hazardous waste.

4

Storage Conditions and Preparation of the Reagents

Storage Conditions

Upon arrival, store the KingFisher Magnetic Beads at +4°C. All the other buffers and reagents included in the KingFisher Pure DNA Blood Kit can be stored at room temperature (15–25°C). The Proteinase K solution is stable at room temperature until the seal of the vial is broken. After being opened, the vial should be stored at -20°C. The reagents are stable for up to three years from the manufacturing date.

Preparation of the Wash Buffers

Add the indicated volume of isopropanol (100%) and ethanol (96–100%) to 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. 98050196) and 384 samples (Cat. No. 98050496)	
	Wash Buffer 1	Wash Buffer 2
Concentrated buffer	50 ml	60 ml
Ethanol (96–100%)	150 ml	180 ml
Total volume	200 ml	240 ml

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

Preparation of the Lysis Buffer A

When purifying DNA from woody, lignified, and/or polyphenol-rich samples, such as branches, twigs, needles, wax-coated leaves (e.g. laurel), and wheat flour, supplement the Lysis Buffer A with polyvinylpyrrolidone (PVP) at a 2% (w/v) final concentration.

When purifying DNA from seeds (e.g. *Brassica napus*), supplement the Lysis Buffer A with dithiothreitol (DTT) at a 40 mM final concentration.

5

Protocols and Pipetting Instructions

Before beginning the DNA purification protocol, carefully read through the *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. No. N07669), the *Thermo Scientific™ KingFisher™ Duo User Manual* (Cat. No. N12420), or the *Thermo Scientific™ KingFisher™ mL User Manual* (Cat. No. 1508260), and the *Thermo Scientific™ BindIt™ Software for KingFisher Instruments version 3.2 User Manual* (Cat. No. N07974).

BindIt Software 3.2 protocols can be found 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. The paramagnetic beads have a tendency to sediment relatively quickly.

Sample Material and Storage

Use ≤ 50 mg of fresh or frozen plant material as sample material. To minimize DNA degradation, avoid repeated freezing and thawing of the samples.

Using young plant samples, and/or if possible keeping plants for 12 h in darkness before collecting the samples, reduces the polysaccharide and polyphenolic contents, which may interfere in downstream applications. Appropriate sample storage is essential for reproducibility and high DNA

yields. The yields of DNA may vary depending on sample age, type of sample, and storage conditions. With dried material, use only one fifth of the starting material compared to the fresh sample material.

Homogenization of Sample Material

Efficient homogenization of the sample material is an essential step before DNA purification in order to gain a good yield of high-quality DNA. Plant tissue can be homogenized, for example, with a pestle, using steel beads or with commercial homogenizers, of which high-throughput homogenizers provide a suitable method for handling 96 samples simultaneously. The homogenization step must disrupt the structures of the starting material rapidly and completely in order to ensure a high yield of DNA.

When purifying DNA from woody, lignified, and/or polyphenol-rich samples, such as branches, twigs, needles, wax-coated leaves (e.g. laurel), and wheat flour, supplement the Lysis Buffer A with polyvinylpyrrolidone (PVP) at a 2% (w/v) final concentration.

When purifying DNA from seeds (e.g. *Brassica napus*), supplement the Lysis Buffer A with dithiothreitol (DTT) at a 40 mM final concentration.

Homogenize ≤ 50 mg of fresh plant sample (or up to 10 mg if dried samples are used). After homogenization, add 350 µl of Lysis Buffer A to a sample and vortex for 20 s. Add 50 µl of Lysis Buffer B to the sample. If RNase A treatment is needed to reduce the amount of RNA in the sample, add 20 µl of RNase A to each sample. RNase A treatment is recommended for the samples containing large amounts of RNA. Vortex the samples for 20 s and incubate at 65°C for 10 min with occasional vortexing or shaking.

To clear the plant lysate, add 130 µl of Precipitation Solution and mix by inverting the sample 3 times followed by incubation on ice for 5 min. Centrifuge the sample for 5 min (20,000 x g). Transfer 400 µl of supernatant to a Thermo Scientific™ Microtiter™ deep well 96 plate and begin the purification of DNA using the KingFisher Flex or KingFisher Duo. Refer to the detailed instructions below.

Instructions for KingFisher Flex with 96 Deep Well Plates

These instructions are intended for DNA purification from 400 µl of plant lysate, using the KingFisher Pure DNA Plant Kit (Cat. No. 98050196 or 98050496) and the KingFisher Flex with Microtiter deep well 96 plates.

When using the KingFisher Flex and the KingFisher Pure DNA Plant Kit for the first time, prepare the Wash Buffer 1 and Wash Buffer 2. For more instructions, refer to "Preparation of the Wash Buffers" on page 15.

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).

1. Homogenize the samples according to the instructions in "Homogenization of Sample Material" on page 18.

2. Take four empty Microtiter deep well 96 plates and two Thermo Scientific™ KingFisher™ Flex 96 KF plates.

Add the following reagents to the Sample plate and leave the plate at room temperature while the other plates are being filled.

3. Fill the **plates** as follows.

Plate number	Plate type	Plate name	Content	Reagent volume per well
1	Microtiter deep well 96 plate	Sample	Plant lysate KingFisher Magnetic Beads* Ethanol	400 µl 25 µl 400 µl
2		Wash 1	Wash Buffer 1	900 µl
3		Wash 2_1	Wash Buffer 2	800 µl
4		Wash 2_2	Wash Buffer 2	800 µl
5	KingFisher Flex 96 KF plate	Elution	Elution Buffer	150 µl

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

4. 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).

5. Start the PURE_DNAPlant_Flex96 protocol with the KingFisher Flex 96 and load the plates as instructed on the KingFisher Flex 96 instrument display.

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 3.2 to the KingFisher Flex. Start the PURE_DNAPlant_Flex96 protocol. Insert the Tip Plate and the filled plates into the instrument as indicated 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_DNAPlant_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 version 3.2 User Manual*.

6. 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 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 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 and Deep Well 96 Plate

These instructions are intended for DNA purification from 400 µl of plant lysate, using the KingFisher Pure DNA Plant Kit (Cat. No. 98050196 or 98050496) and the KingFisher Duo with Microtiter deep well 96 plates.

When using the KingFisher Duo and the KingFisher Pure DNA Plant Kit for the first time, prepare the Wash Buffer 1 and Wash Buffer 2. For more instructions, refer to "Preparation of the Wash Buffers" on page 15.

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).

1. Homogenize the samples according to the instructions in "Homogenization of Sample Material" on page 18.

2. Prepare the **Plant DNA plate** (i.e. a Microtiter deep well 96 plate).

Add the following reagents to the rows. Note that row B is reserved for the tip comb and should be left *empty*. Note that rows C, D, and E are also left *empty*.

Plate name and type	Row	Rpw name	Content	Sample/reagent volume per well
Plant DNA plate Microtiter deep well 96 plate	A	Sample	Homogenized supernatant KingFisher Magnetic Beads* Ethanol	400 µl 25 µl 400 µl
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Empty	Empty	Empty
	F	Wash 1	Wash Buffer 1	900 µl
	G	Wash 2_1	Wash Buffer 2	800 µl
	H	Wash 2_2	Wash Buffer 2	800 µl

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

3. 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

4. Place a Thermo Scientific™ KingFisher™ Duo 12-tip comb into **row B** on a **Plant DNA plate**.

5. Start the PURE_DNAPlant_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 3.2 to the KingFisher Duo. Start the PURE_DNAPlant_Duo protocol. Insert the Plant 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_DNABlood_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 version 3.2 User Manual*.

6. 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 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 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 is kept within the limits specified in the *KingFisher Flex User Manual* or the *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. The amount of beads in the wells 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 the Elution Buffer can be modified depending on user requirements concerning the purified DNA concentration. The final DNA concentration in the Elution Buffer may increase if the purified DNA is eluted into a smaller than recommended volume of the buffer, but this can slightly reduce the overall DNA yield. The modifications of the elution step must be done in BindIt Software 3.2 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 volumes of Elution Buffer in the KingFisher instruments. The Elution Buffer should cover the KingFisher Magnetic Beads completely, and any possible sedimented magnetic beads should be completely resuspended. In addition, the volume of the 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.

A

Troubleshooting

Problem	Possible cause and actions
Low DNA yield	Insufficient homogenization of plant material. To disrupt the cell wall, it is important to homogenize the sample thoroughly. Make sure that ethanol was added to Wash Buffer 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on page 15. Do not let the KingFisher Magnetic Beads dry as this may result in lower elution efficiency. Prolonged storage or repeated freeze/thaw cycles of the sample material may reduce the DNA yield. There should be an adequate volume of the Elution Buffer to cover the KingFisher Magnetic Beads completely during the elution step. 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	Insufficient washing causes impurities in the Elution Buffer.
RNA contamination	Carry out the RNase A treatment step described in the purification procedure.

Continued

Cont.

Problem	Possible cause and actions
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. Improper lysis may also cause problems collecting the KingFisher Magnetic Beads. If the KingFisher Magnetic Beads are inefficiently collected from the Elution Buffer, the addition of a small amount of detergent (e.g. Tween™ 20) may improve the results. Alternatively, centrifuge the eluates or place them on a magnet for a few minutes to collect the residual beads at the bottom of the well. Carryover of the KingFisher Magnetic Beads does not affect most downstream processes. KingFisher Magnetic Beads that occasionally remain attached to the tip combs at the end of the process do not affect the 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.
Inhibition of downstream enzymatic reactions	If the purified DNA contains residual salt, use the correct order for the Wash Buffers. Always wash the KingFisher Magnetic Beads with Wash Buffer 1 first and then proceed with Wash Buffer 2.

B

Ordering Information

Table B-1. KingFisher Pure DNA Plant Kits

Cat. No.	Product	Package size
98050196	KingFisher Pure DNA Plant Kit	96
98050496	KingFisher Pure DNA Plant 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
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
97003520	KingFisher Duo elution strip	40 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

Notes

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