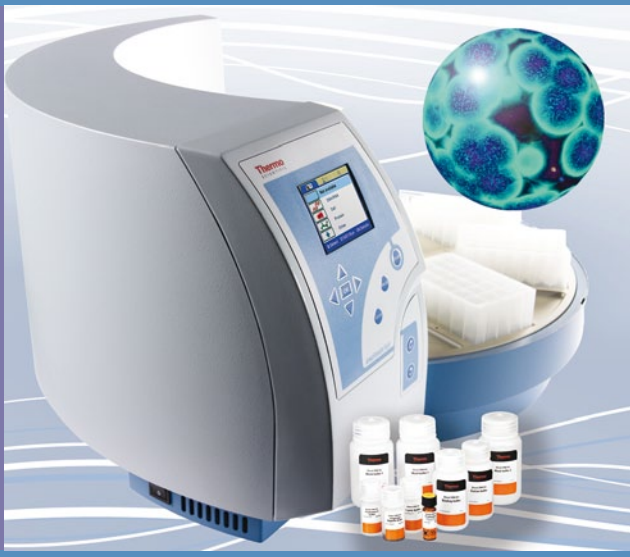
Thermo Scientific KingFisher Cell and Tissue DNA Kit

Instruction Manual

Rev. 1.2



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Thermo Scientific KingFisher Cell and Tissue DNA Kit

Instruction Manual

Rev. 1.2, Cat. no. N11997

Thermo SCIENTIFIC

Contents

Chapter 1
Kit Content

Table 1-1. Thermo Scientific KingFisher Cell and Tissue DNA Kit

Item	KingFisher® Cell and Tissue DNA Kit, 1 x 96
Cat. No.	97030196
Package size	1 x 96 samples
KingFisher Magnetic Beads	2.6 ml
Proteinase K	1 vial
Proteinase K Buffer	3.6 ml
Lysis Buffer	25 ml
Binding Buffer	40 ml
Wash Buffer 1	65 ml
Wash Buffer 2	65 ml
Wash Buffer 3	100 ml
Elution Buffer	20 ml

The KingFisher Cell and Tissue DNA Kit (Cat. No. 97030196) is intended for the purification of cell and tissue samples using the Thermo Scientific KingFisher Flex with a 96 deep well head or the Thermo Scientific KingFisher Duo with a 12-pin head or Thermo Scientific KingFisher mL and a lysed sample volume of 225 µl.

Equipment and reagents to be supplied by the user:

- RNase A (optional)
- Magnetic particle processor

Table 1-2. Thermo Scientific KingFisher magnetic particle processors

Cat. No.	Product
5400000	KingFisher magnetic particle processor
5400050	KingFisher mL magnetic particle processor
5400100	KingFisher Duo magnetic particle processor
5400630	KingFisher Flex magnetic particle processor with 96 deep well head
5400640	KingFisher Flex magnetic particle processor with 24 deep well head
Discontinued	KingFisher 96 magnetic particle processor

Table 1-3. Thermo Scientific 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, non sterile	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 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
97003510	KingFisher Duo 6-tip combs and KingFisher 24 deep well plate (12 pcs of 24 deep well plates, each including 4 tips combs)	48 pcs
97003520	KingFisher Duo elution strip	40 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate (tips combs, plates and elution strips for 96 samples)	1 box

Table 1-5. Thermo Scientific KingFisher mL consumables

Cat. No.	Product	Package size
97002111	KingFisher mL tip comb	800 pcs
97002121	KingFisher mL tube	20 x 45 pcs
97002131	KingFisher mL combi (tubes and tip combs for 60 samples)	60
97002141	KingFisher mL combi (tubes and tip combs for 240 samples)	240

Table 1-6. Thermo Scientific KingFisher consumables

Cat. No.	Product	Package size
97002070	KingFisher tip comb	50 pcs
97002080	KingFisher plate 100 µl	50 pcs
97002084	KingFisher plate 200 µl	50 pcs
97002090	KingFisher plastics 100 µl 8-pack, 8 plates and 8 tip combs	1 box
97002094	KingFisher plastics 200 µl 8-pack, 8 plates and 8 tip combs	1 box

Chapter 2
Product Description

Introduction

The KingFisher Cell and Tissue DNA Kit is designed for rapid automated purification of DNA from cell or bacterial cultures and tissues using KingFisher instruments. DNA purified using the KingFisher Cell and Tissue DNA Kit is of high quality and free of protein, 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 in several other enzymatic reactions.

Intended use

The KingFisher Cell and Tissue DNA Kit is developed for the purification of DNA from cell cultures, tissue samples and bacterial cultures using paramagnetic particles. The purification process requires no phenol/chloroform extraction or alcohol precipitation and needs very little hands-on time. The reagents and specific plastic consumables are designed to work with the KingFisher Flex, KingFisher Duo or KingFisher mL magnetic particle processors as part of an integrated system. The KingFisher Cell and Tissue DNA 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 Cell and Tissue DNA Kit for any particular use, because the performance of the kits has not been validated for any specific organism.

Principle and procedure

The KingFisher Cell and Tissue DNA 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. Samples are lysed in the Lysis Buffer and then sedimented after a short centrifugation step. The cleared lysates are transferred to Thermo Scientific KingFisher plates for processing with a Thermo Scientific KingFisher magnetic particle processor. DNA binds to the surface of the Thermo Scientific KingFisher Magnetic Beads in the presence of a chaotropic salt. The following effective wash steps dispose of proteins, cell debris and any residual contaminants, while the DNA bound to the KingFisher Magnetic Beads is transferred through the wash steps. Three different Wash Buffers are used. Although purified DNA is only rinsed rapidly in the Wash Buffer 3, this step considerably improves the purity of the DNA. High-quality DNA is eluted into the Elution Buffer and is ready for subsequent downstream processes, such as enzymatic reactions. Heat incubation is included in the elution step when using the KingFisher Flex or KingFisher Duo.

Kit specifications

The KingFisher Cell and Tissue DNA Kit is designed for rapid automated preparation of highly pure DNA from cells and tissues using Thermo Scientific KingFisher magnetic particle processors. The approximate processing time is 25 minutes for the purification of 96 samples in the KingFisher Flex and 12 samples in the KingFisher Duo or for the purification of 15 samples in the KingFisher mL. The obtained DNA can be used directly in various downstream applications.

Suitable sample materials for the KingFisher Cell and Tissue DNA Kit are up to 20 mg of tissue, up to 1 x 10^7 of cells, or up to 1 ml of bacteria cultured overnight. It is possible to purify up to 20 µg of DNA depending on the sample and obtain an A_{260}/A_{280} ratio of ≥ 1.6–1.9 per sample. The yields of acquired purified DNA depend on the sample type and the method of sample storage.

The KingFisher Cell and Tissue DNA Kit can be processed at room temperature excluding the lysis step

with Proteinase K treatment, which occurs outside the KingFisher instrument and requires heating. Additionally, heating of the elution step may increase the DNA yield by approximately 15–20%.

The KingFisher Magnetic Beads are highly reactive, superparamagnetic beads. The binding capacity is 0.4 µg of DNA per 1 µl of bead suspension.

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. Use only Thermo Scientific KingFisher plastic consumables. 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 into the plates according to the corresponding instructions. Dispensing can be done 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 optimized protocols for the Thermo Scientific KingFisher Kits. It is also possible to transfer the defined protocol onto the onboard software and run it directly from the instrument. The KingFisher instruments offer a rapid and automated solution for complicated and time-consuming purification processes without risk of carryover or cross contamination, resulting in high-purity DNA.

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 Cell and Tissue DNA Kit is optimized and ready for use with KingFisher systems.

The 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.

Table 2-1. Overview of Thermo Scientific KingFisher systems

	KingFisher Flex		**KingFisher Duo**	
	96 format	**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 (lysed sample volume 225 µl with the KingFisher Cell and Tissue DNA Kit)	Up to 24 samples per run	Up to 12 samples per run	Up to 6 samples per run
Magnet heads	96 interchangeable formats for PCR plate, KingFisher Flex 96 KF plate, Microtiter deep well 96 plate	24 format for KingFisher 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), Microtiter deep well 96 plate (50–1000 µl), 96 well PCR plate, skirted (20–100 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 in room temperature	

Table 2-1. Overview of Thermo Scientific KingFisher systems

	KingFisher mL	KingFisher
Processing volume	50–1000 µl	20–200 µl
Capacity	Up to 15 samples per run (lysed sample volume 225 µl with the KingFisher Cell and Tissue DNA Kit)	Up to 24 samples per run
Magnet heads	15 format	24 format
Plates	KingFisher mL tube, special tube strip with 1 x 5 tubes (50–1000 µl)	KingFisher plate 100 or 200 µl (20–100 µl or 20–200 µl)
Tip combs	KingFisher mL tip comb, 1 x 5 format	KingFisher tip comb, 1 x 12 format
Heating temperature	No heating available	No heating available

The BindIt Software 3.2 protocols optimized for the KingFisher Cell and Tissue DNA Kit are available for the KingFisher Flex 96, the KingFisher Duo and the KingFisher mL instruments. BindIt Software 3.2 protocols for the Thermo Scientific KingFisher and the Thermo Scientific KingFisher 96 are available on request. For more information, contact your local authorized distributor.

Product Description
KingFisher magnetic particle processors

Chapter 3
Safety Information

The following components of the KingFisher Cell and Tissue DNA Kit contain hazardous contents (Table 3-1).

Wear a laboratory coat, disposable gloves and goggles, and follow the safety instructions given 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
Binding Buffer	Sodium perchlorate < 20% and ethanol < 50%	Flammable. Harmful if swallowed. Keep away from food, drink and animal feed. Do not inhale dust. Immediately remove all contaminated clothing.
Wash Buffer 1	Sodium perchlorate < 15% and ethanol < 24%	Flammable. Harmful if swallowed. Keep away from food, drink and animal feed. Do not inhale dust. Immediately remove all contaminated clothing.
Wash Buffer 2	Sodium perchlorate < 15% and ethanol < 24%	Flammable. Harmful if swallowed. Keep away from food, drink and animal feed. Do not inhale dust. Immediately remove all contaminated clothing.
Proteinase K	Lyophilized Proteinase K	Irritating to eyes, respiratory system and skin. May cause sensitization by inhalation. Do not inhale dust. Avoid contact with skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

Chapter 4

Storage Conditions and Preparation of the Working Solution

Storage conditions

All buffers and reagents included in the KingFisher Cell and Tissue Kit can be stored at room temperature (20–25°C) and are stable for up to one year from the manufacturing date. The buffers are ready for use.

Preparation of the Proteinase K working solution

To prepare the Proteinase K working solution for the KingFisher Cell and Tissue DNA Kit, add 2.6 ml of Proteinase K Buffer to the vial of the lyophilized Proteinase K.

The working solution should be stored at -20°C in aliquots. Repeated freezing and thawing should be avoided.

Storage Conditions and Preparation of the Working Solution
Preparation of the Proteinase K working solution

Chapter 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 for the KingFisher and the KingFisher 96 can be obtained on request.

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 or tubes in order to ensure a high consistency between the wells or tubes. To gain complete resuspension of the beads, shake the container vigorously or vortex briefly. The KingFisher Magnetic Beads have a tendency to sediment relatively quickly in the Binding Buffer. Once a premixture of the beads and the Binding Buffer has been made, the mixture should be used immediately to avoid the risk of transferring variable amounts of the beads to the respective wells or tubes.

Lysing of samples

Use a maximum of 1 x 10^7 of cultured cells, 1 ml of bacteria cultured overnight or 20 mg of tissue per sample.

Efficient lysis of the sample is an essential step before DNA purification in order to gain a good yield of high-quality DNA. The lysis step must disrupt the

structures of the sample material completely in order to ensure a high yield of DNA.

Lysing of cells

To lyse the *cells*, centrifuge the sample approximately for 3 minutes at 1200 rpm to pellet the cells to the bottom of the tube. Remove the supernatant and add 200 µl of Lysis Buffer and 25 µl of Proteinase K working solution to each sample. If the Proteinase K working solution has been added to the Lysis Buffer to form a premixture, the solution should be used immediately. Mix the samples *thoroughly* by pipetting up and down *several times* depending on the sample, and vortex for 30 seconds or until the samples are lysed. This is an essential step in the purification process and should be done carefully. Briefly centrifuge the samples and incubate at 70°C for 10–15 minutes. Occasional mixing during the incubation may enhance the lysis. Follow the instructions below for clearing of the lysis mix. Before clearing of the lysis, an optional RNase A treatment can be done (see below).

Lysing of tissue samples

To lyse the *tissue samples*, add 200 µl of Lysis Buffer and 25 µl of Proteinase K working solution to each sample. If the Proteinase K working solution has been added to the Lysis Buffer to form a premixture, the solution should be used immediately. Vortex the samples for 15 seconds, centrifuge briefly and incubate at 56°C until the samples are lysed. Depending on the tissue sample size and structure, lysing should take a minimum of one hour, or even overnight. Follow the instructions below for clearing of the lysis mix. Before clearing of the lysis, an optional RNase A treatment can be done (see below).

RNase A treatment

RNase A treatment is recommended for the samples containing large amounts of RNA or if RNA disturbs downstream applications. Add RNase A (not included) to the lysed sample at a final concentration of 1.8 mg/ml in order to reduce the amount of RNA in the sample.

Incubation should be performed in accordance with the enzyme manufacturer's instructions.

To clear the lysate from debris, centrifuge the samples for 5 minutes (6000 x g). Transfer the supernatant to a new tube, a Thermo Scientific Microtiter deep well 96 plate or a Thermo Scientific KingFisher mL tube strip, and begin the purification of DNA using the KingFisher Flex or the KingFisher mL. See the detailed instructions below.

Instructions for KingFisher Flex with 96 deep well plates for DNA purification from 225 µl of lysed cell or tissue samples

These instructions are for the DNA purification from 225 µl of lysed cell or tissue samples using the KingFisher Cell and Tissue Kit (Cat. No. 97030196, 1 x 96) and the KingFisher Flex with Microtiter deep well 96 plates.

When using the KingFisher Cell and Tissue DNA Kit for the first time, prepare the working solution of Proteinase K. For more instructions, refer to "Preparation of the Proteinase K working solution" on page 19.

1. Lyse the samples according to the instructions given in "Lysing of samples" on page 21. Perform the RNase A treatment (optional). The instructions for the treatment are given in "RNase A treatment" on page 22.

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

3. Prepare four Microtiter deep well 96 plates and one KingFisher Flex 96 KF plate as indicated in the table below. Resuspend the KingFisher Magnetic Beads well (e.g., by vortexing) before transferring them from the bottle.

Plate number	Plate type	Plate name	Content	Reagent volume per well
1	Microtiter deep well 96 plate	Sample	Lysed sample	225 µl
			KingFisher Magnetic Beads	25 µl
			Binding Buffer	360 µl
2	Microtiter deep well 96 plate	Wash 1	Wash Buffer 1	600 µl
3	Microtiter deep well 96 plate	Wash 2	Wash Buffer 2	600 µl
4	Microtiter deep well 96 plate	Wash 3	Wash Buffer 3	800 µl
5	KingFisher Flex 96 KF plate	Elution	Elution Buffer	150 µl

4. Place a Thermo Scientific KingFisher Flex 96 tip comb for deep well magnets on a **Tip Plate** (that is, an empty KingFisher Flex 96 KF plate).

5. Start the KF_TissueDNA_Flex96 protocol with the KingFisher Flex 96 and load the plates.

Switch on the KingFisher Flex and make sure that you are using the KingFisher Flex 96 deep well head and heating block. Connect the PC with BindIt Software 3.2 to the KingFisher Flex. Start the KF_TissueDNA_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 begin.

When the KingFisher Flex is to be run as a standalone instrument, transfer the KF_TissueDNA_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. 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 turn off the instrument. Store the purified DNA accordingly. The purified DNA is ready for use in downstream applications.

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

Summary of plate contents

Table 5-1. Summary of plate contents

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Lysed sample	225 µl
			KingFisher Magnetic Beads	25 µl
			Binding Buffer	360 µl
2	Microtiter deep well 96 plate	Wash 1	Wash Buffer 1	600 µl
3	Microtiter deep well 96 plate	Wash 2	Wash Buffer 2	600 µl
4	Microtiter deep well 96 plate	Wash 3	Wash Buffer 3	800 µl
5	KingFisher Flex 96 KF plate	Elution	Elution Buffer	150 µl
6	KingFisher Flex 96 KF plate	Tip Plate		

Instructions for KingFisher Duo with 12-pin magnet head and 96 deep well plates for DNA purification of 225 µl lysed cell or tissue samples

These instructions are for the DNA purification from 225 µl of lysed cell or tissue samples using the KingFisher Cell and Tissue DNA Kit (Cat. No. 97030196) and the KingFisher Duo with a 12-pin magnet head and Microtiter deep well 96 plates.

When using the KingFisher Cell and Tissue DNA Kit for the first time, prepare the working solution of Proteinase K. For more instructions, refer to "Preparation of the Proteinase K working solution" on page 19.

1. Lyse the samples according to the instructions given in "Lysing of samples" on page 21. Perform the RNase A treatment (optional). The instructions for the treatment are given in "RNase A treatment" on page 22.

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

3. Prepare the **Cell and Tissue DNA plate** (Microtiter deep well 96 plate).

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

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Cell and Tissue DNA plate	A	Sample	Lysed sample	225 µl
Microtiter deep well 96 plate			KingFisher Magnetic Beads	25 µl
			Binding Buffer	360 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	600 µl
	G	Wash 2	Wash Buffer 2	600 µl
	H	Wash 3	Wash Buffer 3	800 µl

4. 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

5. Place a Thermo Scientific KingFisher Duo 12-tip comb into **row B** on a **Cell and Tissue DNA plate**.

6. Start the KF_TissueDNA_Duo protocol with the KingFisher Duo and load the plate and elution strip.

Switch on the KingFisher Duo and make sure that you are using the KingFisher Duo 12-pin magnet head and heating block. Connect the PC with BindIt Software 3.2 to the KingFisher Duo. Start the KF_TissueDNA_Duo protocol. Insert the Cell and Tissue 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 KF_TissueDNA_Duo protocol to the KingFisher Duo. The instructions for transferring the protocol can be found in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

7. After the run is completed, remove the plate 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 turn off the instrument. Store the purified DNA accordingly. The purified DNA is ready for use in downstream applications. The final DNA concentration in the Elution Buffer may increase if the purified DNA is eluted into a smaller volume of the buffer than is recommended, but this can slightly reduce the overall DNA yield.

Summary of plate and elution strip contents

Table 5-2. Summary of plate and elution strip contents

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Cell and Tissue DNA plate	A	Sample	Lysed sample	225 µl
Microtiter deep well 96 plate			KingFisher Magnetic Beads	25 µl
			Binding Buffer	360 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	600 µl
	G	Wash 2	Wash Buffer 2	600 µl
	H	Wash 3	Wash Buffer 3	800 µl
Elution strip		Elution	Elution Buffer	100 µl

Instructions for KingFisher mL for DNA purification from 225 µl of lysed cell or tissue samples

These instructions are for the DNA purification from 225 µl of lysed cell or tissue samples using the KingFisher Cell and Tissue Kit (Cat. No. 97030196) and the KingFisher mL.

When using the KingFisher Cell and Tissue DNA Kit for the first time, prepare the working solution of Proteinase K. For more instructions, refer to "Preparation of the Proteinase K working solution" on page 19.

A tube strip tray in the KingFisher mL can contain up to 15 separate KingFisher mL tube strips, and one sample processing uses one tube strip with five tubes. The orientation of the tube strip is fixed. Note that the tube strips have to be positioned so that the slip ends are facing left. One tip comb with five tips is used for processing five samples at a time.

1. Lyse the samples according to the instructions given in "Lysing of samples" on page 21. Perform the RNase A treatment (optional). The instructions for the treatment are given in "RNase A treatment" on page 22.

2. Place empty KingFisher mL tube strips on a tube strip tray. Prepare the **tubes** (that is, starting from the first tube at the slip end of a tube strip). Add the following reagents to the tubes.

Tube	Tube name	Content	Reagent volume
A	Sample	Lysed sample	225 µl
		KingFisher Magnetic Beads	25 µl
		Binding Buffer	360 µl
B	Wash 1	Wash Buffer 1	600 µl
C	Wash 2	Wash Buffer 2	600 µl
D	Wash 3	Wash Buffer 3	800 µl
E	Elution	Elution Buffer	150 µl

3. Prepare the KingFisher mL for the run.

Switch on the KingFisher mL and insert the tray into the instrument. Insert the tip combs into their slots and close the front lid.

4. Start the KF_TissueDNA_mL protocol with the KingFisher mL.

Connect the PC with BindIt Software 3.2 to the KingFisher mL. Start the KF_TissueDNA_mL protocol.

When the KingFisher mL is to be run as a standalone instrument, transfer the KF_TissueDNA_mL protocol to the KingFisher mL. 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*.

5. After the run is completed, remove the tube strips and store the purified DNA.

When the protocol is completed, remove the tubes and turn off the instrument. Store the purified DNA accordingly. The purified DNA is ready for use in downstream applications.

To increase the DNA yield, the Elution Buffer can be prewarmed to 55°C and dispensed into the Elution tubes during an additional pause step in the protocol before the elution step.

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

Summary of tube contents

Table 5-3. Summary of tube contents

Tube	Tube name	Content	Sample/reagent volume
A	Sample	Lysed sample	225 µl
		KingFisher Magnetic Beads	25 µl
		Binding Buffer	360 µl
B	Wash 1	Wash Buffer 1	600 µl
C	Wash 2	Wash Buffer 2	600 µl
D	Wash 3	Wash Buffer 3	800 µl
E	Elution	Elution Buffer	150 µl

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, and the value for DNA should be ≥ 1.7–2.0. A Thermo Scientific NanoDrop can be used for the direct measurement of DNA without diluting the sample.

It is recommended that A_{320} correction be 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 beads.

Use these calculations to measure the DNA content:

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

Chapter 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, KingFisher Duo User Manual* or *KingFisher mL User Manual* to avoid spillover and to keep performance at the most efficient level.

Handling of magnetic beads

The KingFisher Magnetic Beads have a tendency to sediment relatively quickly in the Binding Buffer. Once a premixture of the beads and the Binding Buffer has been made, the mixture should be used immediately 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 and wash steps

The binding between the DNA and the KingFisher Magnetic Beads is strong in the presence of a chaotropic salt, but chaotropic salts are not present in the Wash Buffer 3 and accordingly the binding is weak. Avoid strong mixing speeds and releasing the beads into the Wash Buffer 3 in order to minimize the loss of DNA during the wash step. A short wash and a slow mixing speed without releasing the beads into the buffer are recommended.

Elution step

Carryover of ethanol to the Elution Buffer causes impurities in the Elution Buffer and may affect some downstream applications. To remove traces of ethanol, make sure that there is a wash step (e.g., washing without releasing the beads) or the drying step before the elution step is long enough. There is a delicate balance between complete removal of the ethanol and loss of DNA.

The volume of the Elution Buffer can be modified depending on the 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 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 Thermo Scientific KingFisher instruments

KingFisher instrument	Elution volume
KingFisher	20–200 µl
KingFisher mL	50–1000 µl
KingFisher Duo with 12-pin magnet head	30–130 µl
KingFisher Duo with 6-pin magnet head	200–5000 µl
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 96 well head, elution in a KingFisher Flex 96 KF plate	20–250 µl
KingFisher Flex with 24 deep well head	200–5000 µl

To gain a maximal 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 magnetic-bead pellet 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.

Elution can be conducted at room temperature. The elution at 72°C in the KingFisher Flex and KingFisher Duo or at 55°C outside the KingFisher instrument will, however, increase the yield by approximately 15–20%. When using the KingFisher instrument without a heating option, the Elution Buffer can be prewarmed to 55°C and dispensed into the Elution tubes or wells during an additional pause in the protocol before the elution step.

If some KingFisher Magnetic Beads remain in the Elution Buffer, centrifuge the Elution plate briefly or place it on a magnet for a few minutes to collect the residual beads at the bottom of the well and transfer the supernatant to a new tube.

RNase A treatment is recommended if the purity of the DNA is not sufficient for downstream applications with the normal protocol.

Appendix A
Troubleshooting

Table A-1. Troubleshooting guide

Problem	Possible cause and actions
Low DNA yield	There should be an adequate volume of the Elution Buffer to cover the KingFisher Magnetic Beads completely during the elution step. Do not let the KingFisher Magnetic Beads dry, as this may result in lower elution efficiency. Heating during the elution step or using a preheated Elution Buffer enhances the release of the DNA from the KingFisher Magnetic Beads and the yield of the purified DNA. Efficient lysis of the cells or tissue increases the DNA yield. Prolonged storage of the sample material may reduce the DNA yield. If the KingFisher instrument mixes too strongly during the wash 3, it may cause partial elution of DNA already in the Wash Buffer 3. Use only Thermo Scientific KingFisher plates, tubes or 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 of the DNA. Insufficient washing causes impurities in the Elution Buffer. Wash Buffer 2 contains ethanol. Carryover of the buffer may cause unsatisfactory performance in downstream applications. Carryover of the KingFisher Magnetic Beads to the Elution Buffer may affect the A_{260}/A_{280} ratio. Make sure that the KingFisher Magnetic Beads do not affect the measurement by centrifuging the samples or placing 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 cause problems in most downstream processes.

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Problem	Possible cause and actions
Magnetic particles remaining in the lysed 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. The samples can, for example, be diluted into 1 x PBS. 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., 0.02% Tween 20) may improve the results. Carryover of the KingFisher Magnetic Beads to the Elution Buffer may affect the A_{260}/A_{280} ratio. Refer to "Low purity" on page 37. 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.

Appendix B
Ordering Information

Table B-1. Thermo Scientific KingFisher Cell and Tissue DNA Kits

Cat. No.	Product	Package size
97030196	KingFisher Cell and Tissue DNA Kit	1 x 96

Table B-2. Thermo Scientific 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, non sterile	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. Thermo Scientific 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 combs and KingFisher 24 deep well plate (12 pcs of 24 deep well plates, each including 4 tips combs)	48 pcs
97003520	KingFisher Duo elution strip	40 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate (tips combs, plates and elution strips for 96 samples)	1 box

Table B-4. Thermo Scientific KingFisher mL consumables

Cat. No.	Product	Package size
97002111	KingFisher mL tip comb	800 pcs
97002121	KingFisher mL tube	20 x 45 pcs
97002131	KingFisher mL combi (tubes and tip combs for 60 samples)	60
97002141	KingFisher mL combi (tubes and tip combs for 240 samples)	240

Table B-5. Thermo Scientific KingFisher consumables

Cat. No.	Product	Package size
97002070	KingFisher tip comb	50 pcs
97002080	KingFisher plate 100 µl	50 pcs
97002084	KingFisher plate 200 µl	50 pcs
97002090	KingFisher plastics 100 µl 8-pack, 8 plates and 8 tip combs	1 box
97002094	KingFisher plastics 200 µl 8-pack, 8 plates and 8 tip combs	1 box

Notes

Notes

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