Thermo Scientific KingFisher Blood DNA Kit

Instruction Manual Rev. 1.2





Thermo Scientific KingFisher Blood DNA Kit

Instruction Manual

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Chapter 1 Kit Content

ltem	KingFisher® Blood DNA Kit, 1 x 96
Cat. No.	97010196
Package size	1 x 96 samples
KingFisher Magnetic Beads	2.6 ml
Proteinase K	1 vial
Proteinase K Buffer	3 ml
Lysis Buffer	11 ml
Binding Buffer	45 ml
Wash Buffer 1	2 x 85 ml
Wash Buffer 2	110 ml
Elution Buffer	20 ml

Table 1-1.	Thermo Scientific KingFisher Blood DNA Kit

The KingFisher Blood DNA Kit (Cat. No. 97010196) is intended for the purification of blood 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 sample volume of 250 μ l. The kit is also suitable for the KingFisher Flex with a 24 deep well head or the KingFisher Duo with a 6-pin head and a sample volume of 3 ml. When a large sample volume is used, the kit can handle 9 samples.

Equipment and reagents to be supplied by the user:

- 80% ethanol (EtOH), molecular biology grade
- Magnetic particle processor

Table 1-2.	Thermo Scientific KingFisher magnetic particle processors	
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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

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 1-4. Thermo Scientific KingFisher Duo consumables

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 μ l 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

Kit Content

Chapter 2 Product Description

Introduction

The KingFisher Blood DNA Kit is designed for rapid automated purification of DNA from whole blood samples using KingFisher instruments. DNA can be purified from fresh or frozen blood treated with EDTA (ethylenediaminetetraacetic acid) or citrate. DNA purified using the KingFisher Blood DNA 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 in several other enzymatic reactions.

Intended use The KingFisher Blood DNA Kit is developed for the purification of DNA from whole blood samples 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 Blood 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 Blood 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 Blood 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. The first step of the protocol lyses the blood cells, after which the DNA can bind 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. Two different Wash Buffers are used, followed by a rapid rinse in the third Wash Buffer or an air drying step, which 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 Blood DNA Kit is designed for rapid automated preparation of highly pure DNA from whole blood using Thermo Scientific KingFisher magnetic particle processors. Excluding a dispense step requiring the addition of the Binding Buffer, the approximate processing time is 35 minutes for the purification of 96 samples in the KingFisher Flex and 12 samples in the KingFisher Duo and 45 minutes for the purification of 15 samples in the KingFisher mL when the sample volume is 250 µl. The obtained DNA can be used directly in various downstream applications.

Fresh or frozen blood treated with either EDTA or citrate can be used. The procedure is optimized for sample volumes of 250 μ l or 3 ml but the protocol can be scaled to sample volumes between these volumes.

The kit provides reagents for the purification of 3–10 μ g of pure DNA from 250 μ l of whole blood with an A₂₆₀/A₂₈₀ ratio of \geq 1.7–2.0. The DNA yields depend on the sample type, the sample storage, and the number of white blood cells, if purified from whole blood.

The KingFisher Blood DNA Kit can be processed at room temperature. Heating during the elution step may, however, increase the DNA yield by approximately 15–20%.

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

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. 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 and optimized protocols for the Thermo Scientific KingFisher Kits. It is also possible to transfer the developed 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 μ l. Each system consists of an instrument, specially designed plastic consumables and the easy-to-use BindIt° Software 3.2.

KingFisher magnetic particle processors

The KingFisher Blood 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.

	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 (sample volume 250 µl with the KingFisher Blood DNA Kit)	Up to 24 samples per run (sample volume 3 ml with the KingFisher Blood DNA Kit)	Up to 12 samples per run	Up to 6 samples per run (sample volume 3 ml)
Magnetic head	96 interchangeable 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

Table 2-1. Overview of Thermo Scientific KingFisher systems

KingFisher magnetic particle processors

	KingFisher Flex		KingFisher Duo	
	96 format	24 format	12 format	6 format
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 King	Fisher systems

	KingFisher mL	KingFisher
Processing volume	50–1000 μl	20–200 μl
Capacity	Up to 15 samples per run (sample volume 250 µl with the KingFisher Blood DNA Kit)	Up to 24 samples per run
Magnetic head	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 Blood DNA kit are available for the KingFisher Flex 96 and 24, 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.

Chapter 3 Safety Information

The following components of the KingFisher Blood 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.

Reagent	Hazardous contents	Safety instructions
Lysis Buffer	Guanidine hydrochloride < 45%	Harmful if swallowed. Irritating to eyes and skin. Do not inhale dust.
Binding Buffer	Sodium perchlorate < 30% 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.
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 clothing and gloves.

Table 3-1. Safety precautions

Safety Information

Chapter 4 Storage Conditions and Preparation of the Working Solution

Storage conditions

All buffers and reagents included in the KingFisher Blood DNA Kit can be stored at room temperature $(20-25^{\circ}C)$ 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 Blood DNA Kit, add 2.5 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 KingFisher 96, as well as BindIt Software 3.2 walk-away protocols for the KingFisher Flex, the KingFisher Duo and the KingFisher mL can be obtained on request. Walk-away protocols do not include a dispense step, but the yield and purity of the DNA may be lower than with the normal protocol.

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. Instructions for KingFisher Flex with 96 deep well plates for DNA purification from 250 µl of blood

Instructions for KingFisher Flex with 96 deep well plates for DNA purification from 250 µl of blood

These instructions are for the DNA purification from 250 μ l of blood using the KingFisher Blood DNA Kit (Cat. No. 97010196) and the KingFisher Flex with Thermo Scientific Microtiter deep well 96 plates.

When using the KingFisher Blood 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. Take five empty Microtiter deep well 96 plates and two empty Thermo Scientific KingFisher Flex 96 KF plates.
- 2. Prepare the **Sample plate** (that is, a Microtiter deep well 96 plate).

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

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well	Sample	Blood sample	250 µl
	96 plate		Lysis Buffer	100 µl
			Proteinase K working solution	20 µl

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

Plate number	Plate type	Plate name	Content	Reagent volume per well
2	Microtiter deep well 96 plate	Wash 1_1	Wash Buffer 1	800 µl
3	Microtiter deep well 96 plate	Wash 1_2	Wash Buffer 1	800 µl

Instructions for KingFisher Flex with 96 deep well plates for DNA purification from 250 µl of blood

Plate number	Plate type	Plate name	Content	Reagent volume per well
4	Microtiter deep well 96 plate	Wash EtOH	80% ethanol	800 µl
5	Microtiter deep well 96 plate	Wash 2	Wash Buffer 2	1000 µl
6	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_BloodDNA_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_BloodDNA_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_BloodDNA_Flex 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. Add the KingFisher Magnetic Beads and Binding Buffer to the Sample plate during the dispense step.

When the KingFisher Flex pauses at the dispense step after the lysis step (approximately 5 minutes after starting the protocol run), add the KingFisher Magnetic Beads and Instructions for KingFisher Flex with 96 deep well plates for DNA purification from 250 µl of blood

Binding Buffer separately to the **Sample plate**. Resuspend the KingFisher Magnetic Beads well (e.g., by vortexing) before removing them from the bottle.

Plate number	Plate type	Plate name	Content	Reagent volume per well
1	Microtiter deep well 96 plate	Sample	KingFisher Magnetic Beads	25 µl
	oo plate		Binding Buffer	400 µl

- 7. Place the Sample plate back into the instrument and press **Start**. After the pause, the protocol will continue to the end.
- 8. 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	Sample	Blood sample	250 µl
	96 plate		Lysis Buffer	100 µl
			Proteinase K working solution	20 µl
400 µl of I		er per well	isher Magnetic Be to the Sample plate	
2	Microtiter deep well 96 plate	Wash 1_1	Wash Buffer 1	800 µl
3	Microtiter deep well 96 plate	Wash 1_2	Wash Buffer 1	800 µl
4	Microtiter deep well 96 plate	Wash EtOH	80% ethanol	800 µl
5	Microtiter deep well 96 plate	Wash 2	Wash Buffer 2	1000 µl
6	KingFisher Flex 96 KF plate	Elution	Elution Buffer	150 µl
7	KingFisher Flex 96 KF plate	Tip Plate		

Instructions for KingFisher Flex with 24 deep well plates for DNA purification from 3 ml of blood

Instructions for KingFisher Flex with 24 deep well plates for DNA purification from 3 ml of blood

These instructions are for the DNA purification from 3 ml of blood using the KingFisher Blood DNA Kit (Cat. No. 97010196) and the KingFisher Flex with Thermo Scientific KingFisher Flex 24 deep well plates.

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

- 1. Take eight empty KingFisher Flex 24 deep well plates.
- 2. Prepare the **Sample plates 1 and 2**. The 3 ml blood sample is divided into two different plates on the Sample plate 1 and 2. Pipette 1.5 ml sample into the well on plate 1 and 1.5 ml sample into the corresponding well on plate 2. A 3 ml blood sample is divided, for example, into wells A1 on Sample plate 1 and A1 on Sample plate 2. Add the following reagents to the Sample plates 1 and 2, and leave the plates at room temperature while the other plates are being filled.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	KingFisher Flex 24	Sample 1	Blood sample	1500 µl
	deep well plate		Lysis Buffer	575 µl
	prato		Proteinase K working solution	150 µl
2	KingFisher Flex 24	Sample 2	Blood sample	1500 µl
	deep well plate		Lysis Buffer	575 µl
	•		Proteinase K working solution	150 µl

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

Protocols and Pipetting Instructions

Instructions for KingFisher Flex with 24 deep well plates for DNA purification from 3 ml of blood

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
3	KingFisher Flex 24 deep well plate	Wash 1_1	Wash Buffer 1	4800 µl
4	KingFisher Flex 24 deep well plate	Wash 1_2	Wash Buffer 1	4800 µl
5	KingFisher Flex 24 deep well plate	Wash EtOH	80% ethanol	4800 µl
6	KingFisher Flex 24 deep well plate	Wash 2	Wash Buffer 2	5000 μl
7	KingFisher Flex 24 deep well plate	Elution	Elution Buffer	500 µl

- 4. Place a Thermo Scientific KingFisher Flex 24 tip comb on a **Tip Plate** (that is, an empty KingFisher Flex 24 deep well plate).
- 5. Start the KF_BloodDNA_Flex24 protocol with the KingFisher Flex 24 and load the plates.

Switch on the KingFisher Flex and make sure that you are using the Thermo Scientific KingFisher Flex 24 deep well head and heating block. Connect the PC with BindIt Software 3.2 to the KingFisher Flex. Start the KF_BloodDNA_Flex24 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. Instructions for KingFisher Flex with 24 deep well plates for DNA purification from 3 ml of blood

When the KingFisher Flex is to be run as a standalone instrument, transfer the KF_BloodDNA_Flex24 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. Add the KingFisher Magnetic Beads and Binding Buffer to the Sample plate during the dispense step.

When the KingFisher Flex pauses at the dispense step after the lysis step (approximately after 10 minutes), remove the Sample plates 1 and 2 from the instrument and separately add 75 μ l of KingFisher Magnetic Beads and 2300 μ l of Binding Buffer per well to the Sample plates 1 and 2. Resuspend the KingFisher Magnetic Beads well (e.g., by vortexing) before transferring them from the bottle.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	KingFisher Flex 24 deep well plate	Sample 1	KingFisher Magnetic Beads	75 µl
			Dinding Duffer	0000 1
			Binding Buffer	2300 µl
2	KingFisher Flex 24 deep well plate	Sample 2	KingFisher Magnetic Beads	2300 μl 75 μl

- 7. Place the Sample plates back into the instrument and press **Start**. After the pause, the protocol will continue to the end.
- 8. After the run is completed, remove the plates and store the purified DNA.

Instructions for KingFisher Flex with 24 deep well plates for DNA purification from 3 ml of blood

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.

Protocols and Pipetting Instructions Instructions for KingFisher Flex with 24 deep well plates for DNA purification from 3 ml of blood

Summary of	Table 5-2. Summary of plate contents					
plate contents	Plate number	Plate type	Plate name	Content	Sample/reagent volume per well	
	1	KingFisher Flex 24 deep	Sample 1	Blood sample	1500 µl	
		well plate		Lysis Buffer	575 µl	
				Proteinase K working solution	150 µl	
	2	KingFisher Flex 24 deep	Sample 2	Blood sample	1500 µl	
		well plate		Lysis Buffer	575 µl	
				Proteinase K working solution	150 µl	
	2300 µl o		er per well t		eads per well and tes 1 and 2 during	
	3	KingFisher Flex 24 deep well plate	Wash 1_1	Wash Buffer 1	4800 µl	
	4	KingFisher Flex 24 deep well plate	Wash 1_2	Wash Buffer 1	4800 µl	
	5	KingFisher Flex 24 deep well plate	Wash EtOH	80% ethanol	4800 µl	
	6	KingFisher Flex 24 deep well plate	Wash 2	Wash Buffer 2	5000 μl	
	7	KingFisher Flex 24 deep well plate	Elution	Elution Buffer	500 µl	
	8	KingFisher Flex 24 deep well plate	Tip Plate			

Summary of Table 5-2. Summary of plate contents

Instructions for KingFisher Duo with 12-pin magnet head and 96 deep well plates for DNA purification from 250 µl of blood

These instructions are for the DNA purification from 250 μ l of blood using the KingFisher Blood DNA Kit (Cat. No. 97010196) and the KingFisher Duo with a 12-pin magnet head and Microtiter deep well 96 plates.

When using the KingFisher Blood 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. Take one empty Microtiter deep well 96 plate and one Thermo Scientific KingFisher Duo elution strip.
- 2. Prepare the **Blood DNA plate** (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 and D are left empty.

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Blood DNA plate	А	Sample	Blood sample	250 µl
Microtiter deep well 96 plate			Lysis Buffer	100 µl
			Proteinase K working solution	20 µl
	В	Тір	12-tip comb	Empty
	С	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Wash 1_1	Wash Buffer 1	800 µl
	F	Wash 1_2	Wash Buffer 1	800 µl
	G	Wash EtOH	80% ethanol	800 µl
	Н	Wash 2	Wash Buffer 2	1000 µl

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 **Blood DNA plate**.
- 5. Start the KF_BloodDNA_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_BloodDNA_Duo protocol. Insert the Blood 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_BloodDNA_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.*

6. Add the KingFisher Magnetic Beads and Binding Buffer to row A in the Blood DNA plate during the dispense step.

When the KingFisher Duo pauses at the dispense step after the lysis step (approximately 5 minutes after starting the protocol run), add the KingFisher Magnetic Beads and Binding Buffer separately to row A in the **Blood DNA plate**. 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
Blood DNA plate	А	Sample	KingFisher Magnetic Beads	25 µl
Microtiter deep well 96 plate			Binding Buffer	400 µl

- 7. Place the Blood DNA plate back into the instrument and press **OK**. After the pause, the protocol will continue to the end.
- 8. 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 5-3. Summary of plate and elution strip contents

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well	
Blood DNA plate	А	Sample	Blood sample	250 μl	
Microtiter deep well 96 plate			Lysis Buffer	100 µl	
			Proteinase K working solution	20 µl	
	Dispense step: add 25 μl of KingFisher Magnetic Beads per well and 400 μl of Binding Buffer per well to row A during the pause in the Bindlt protocol.				
	В	Тір	12-tip comb	Empty	
	С	Empty	Empty	Empty	
	D	Empty	Empty	Empty	
	E	Wash 1_1	Wash Buffer 1	800 µl	
	F	Wash 1_2	Wash Buffer 1	800 µl	
	G	Wash EtOH	80% ethanol	800 µl	
	Н	Wash 2	Wash Buffer 2	1000 µl	
Elution strip		Elution	Elution Buffer	100 µl	

Protocols and Pipetting Instructions

Instructions for KingFisher Duo with 6-pin magnet head and 24 deep well plates for DNA purification from 3 ml of blood

Instructions for KingFisher Duo with 6-pin magnet head and 24 deep well plates for DNA purification from 3 ml of blood

These instructions are for the DNA purification from 3 ml of blood using the KingFisher Blood DNA Kit (Cat. No. 97010196) and the KingFisher Duo with a 6-pin magnet head and KingFisher Flex 24 deep well plates.

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

- 1. Take two empty KingFisher Flex 24 deep well plates.
- 2. Prepare the **Blood DNA plates 1 and 2**. Add the following reagents to the Blood DNA plates 1 and 2 (KingFisher Flex 24 deep well plates). The 3 ml blood sample is divided into two different wells on rows A and B on the Blood DNA plate 1. Pipette 1.5 ml sample into the well on row A and 1.5 ml sample into the corresponding well on row B. A 3 ml blood sample is divided, for example, into wells A1 and B1 on the Blood DNA plate 1.

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Blood DNA plate 1	А	Sample 1_1	Blood sample	1500 µl
KingFisher Flex 24 deep well plate			Lysis Buffer	575 µl
			Proteinase K working solution	150 µl
	В	Sample 1_2	Blood sample	1500 µl
			Lysis Buffer	575 µl
			Proteinase K working solution	150 µl
	С	Тір	6-tip comb	Empty
	D	Wash 1_1	Wash Buffer 1	4800 µl

Protocols and Pipetting Instructions

Instructions for KingFisher Duo with 6-pin magnet head and 24 deep well plates for DNA purification from 3 ml of blood

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Blood DNA plate 2	А	Elution	Elution Buffer	500 µl
KingFisher Flex 24 deep well plate	В	Wash 2	Wash Buffer 2	5000 μl
	С	Wash EtOH	80% ethanol	4800 µl
	D	Wash 1_2	Wash Buffer 1	4800 µl

- 3. Place a Thermo Scientific KingFisher Duo 6-tip comb into row C in the Blood DNA plate 1.
- 4. Start the KF_BloodDNA_Duo3ml protocol with the KingFisher Duo and load the plates.

Switch on the KingFisher Duo and make sure that you are using the KingFisher Duo 6-pin magnet head and heating block. Connect the PC with BindIt Software 3.2 to the KingFisher Duo. Start the KF_BloodDNA_Duo3ml protocol. Insert the Blood DNA plates into the instrument as indicated on the KingFisher Duo display and press **OK**. After all the plates have been loaded into the instrument, the protocol will begin.

When the KingFisher Duo is to be run as a standalone instrument, transfer the KF_BloodDNA_Duo3ml 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.*

5. Add the KingFisher Magnetic Beads and Binding Buffer to row A and B in the Blood DNA plate 1 during the dispense step.

When the KingFisher Duo pauses at the dispense step after the lysis step (approximately after 10 minutes),

Instructions for KingFisher Duo with 6-pin magnet head and 24 deep well plates for DNA purification from 3 ml of blood

remove the Blood DNA plate 1 from the instrument and separately add 75 μ l of KingFisher Magnetic Beads and 2300 μ l of Binding Buffer per well to rows A and B in the Blood DNA plate 1. Resuspend the KingFisher Magnetic Beads well (e.g., by vortexing) before transferring them from the bottle.

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Blood DNA plate 1	А	Sample 1_1	KingFisher Magnetic Beads	75 µl
KingFisher Flex 24 deep well plate			Binding Buffer	2300 µl
	В	Sample 1_2	KingFisher Magnetic Beads	75 µl
			Binding Buffer	2300 µl

- 6. Place the Blood DNA plate 1 back into the instrument and press **OK**. After the pause, the protocol will continue to the end.
- 7. 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 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.

Protocols and Pipetting Instructions

Instructions for KingFisher Duo with 6-pin magnet head and 24 deep well plates for DNA purification from 3 ml of blood

Summary of plate contents

Summary of Table 5-4. Summary of plate contents

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Blood DNA plate 1	А	Sample 1_1	Blood sample	1500 µl
KingFisher Flex 24 deep well plate			Lysis Buffer	575 µl
			Proteinase K working solution	150 µl
	В	Sample 1_2	Blood sample	1500 µl
			Lysis Buffer	575 µl
			Proteinase K working solution	150 µl
	Dispense step: add 75 µl of KingFisher Magnetic Beads per well and 2300 µl of Binding Buffer per well to rows A and B during the pause in the Bindlt protocol.			
	С	Тір	6-tip comb	Empty
	D	Wash 1_1	Wash Buffer 1	4800 µl
Blood DNA plate 2	А	Elution	Elution Buffer	500 µl
KingFisher Flex 24	В	Wash 2	Wash Buffer 2	5000 µl
deep well plate	С	Wash EtOH	80% ethanol	4800 µl
	D	Wash 1_2	Wash Buffer 1	4800 µl

Instructions for KingFisher mL for DNA purification from 250 µl of blood

These instructions are for the DNA purification from 250 μ l of blood using the KingFisher Blood DNA Kit (Cat. No. 97010196) and the KingFisher mL.

When using the KingFisher Blood 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 may contain up to 15 separate Thermo Scientific 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. Place empty KingFisher mL tube strips on a tube strip tray. Prepare the **Sample tubes** (that is, starting from the first tube at the slip end of a tube strip). Add the following reagents to the Sample tubes.

Tube	Tube name	Content	Sample/reagent volume
А	Sample	Blood sample	250 µl
		Lysis Buffer	100 µl
		Proteinase K working solution	20 µl
В	Wash 1_1	Wash Buffer 1	800 µl
С	Wash 1_2	Wash Buffer 1	800 µl
D	Wash EtOH	80% ethanol	1000 µl
E	Elution	Elution Buffer	150 µl

2. 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.

Instructions for KingFisher mL for DNA purification from 250 µl of blood

3. Start the KF_BloodDNA_mL protocol with the KingFisher mL.

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

When the KingFisher mL is to be run as a standalone instrument, transfer the KF_BloodDNA_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.

4. Add the KingFisher Magnetic Beads and Binding Buffer to the Sample tubes during the dispense step.

When the KingFisher mL pauses at the dispense step after the lysis step (approximately after 5 minutes), remove the tube strip tray from the instrument and separately add 25 μ l of KingFisher Magnetic Beads and 400 μ l of Binding Buffer to each **Sample tube**. Resuspend the KingFisher Magnetic Beads well (e.g., by vortexing) before transferring them from the bottle.

Tube	Tube name	Content	Sample/reagent volume
A	Sample	KingFisher Magnetic Beads	25 µl
		Binding Buffer	400 μl

- 5. Place the tube strip tray back into the instrument and press **Start**. After the pause, the protocol will continue to the end.
- 6. 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 5-5. Summary of tube contents

Tube	Tube name	Content	Sample/reagent volume
А	Sample	Blood sample	250 µl
		Lysis Buffer	100 µl
		Proteinase K working solution	20 µl

Dispense step: add 25 μ l of KingFisher Magnetic Beads per tube and 400 μ l of Binding Buffer per tube into the Sample tubes (A tubes) during the pause in the Bindlt protocol.

В	Wash 1_1	Wash Buffer 1	800 µl	
С	Wash 1_2	Wash Buffer 1	800 µl	
D	Wash EtOH	80% ethanol	1000 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 $\geq 1.7-2.0$. A Thermo Scientific Nanodrop can be used for direct measurement of DNA without diluting the sample.

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 beads.

Use these calculations to measure the DNA content:

- Concentration of DNA =50 µg/ml x (A₂₆₀ – A₃₂₀) x dilution factor
- *Total amount of DNA* = concentration x volume of sample in ml
- Purity of $DNA = (A_{260} A_{320})/(A_{280} A_{320})$

Reagent specificity and volumes

Handling of

magnetic beads

Chapter 6 General Information

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

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 2 and accordingly the binding is weak. Avoid strong mixing speeds and releasing the beads into the Wash Buffer 2 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 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.

Decontamination and disinfection of sample material

The sample material and the reagents and plastics that have been in contact with the sample material should be decontaminated in order to minimize the risk of contamination. For this purpose, a decontaminant, such as Virkon[®], should be used in accordance with the manufacturer's instructions. Accordingly, the appropriate treatment and/or disposal of waste should be taken care of.

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 blood cells increases the DNA yield.
	Prolonged storage of the sample material may reduce the DNA yield.
	If the mixing is too strong during the wash 2, it may cause partial elution of DNA already in the Wash Buffer 2.
	Use only Thermo Scientific KingFisher plates or tubes 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. Blood can be stored at 2–8°C for approximately two weeks. Freezing of the samples is recommended if stored for long periods.
	Insufficient washing causes impurities in the Elution Buffer.
	Blood clots in the sample cause impurities in the purified DNA.
	Wash Buffer 1 contains ethanol in addition to the specific ethanol wash step. 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 ₂₆₀ 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 affect most downstream processes.

Continued

Problem	Possible cause and actions
Magnetic particles remaining in the lysed sample or elution	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 $A_{\rm 260}/A_{\rm 280}$ ratio. Refer to "Low purity" on page 47.
	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 Blood DNA Kits

Cat. No.	Product	Package size
97010196	KingFisher Blood 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

Ordering Information

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-4. Thermo Scientific KingFisher mL consumables

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 μ l 8-pack, 8 plates and 8 tip combs	1 box
97002094	KingFisher plastics 200 μ l 8-pack, 8 plates and 8 tip combs	1 box

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