Thermo Scientific KingFisher Total RNA Kit

Instruction Manual

Rev. 1.2

Part of Thermo Fisher Scientific

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Instruction Manual

Rev. 1.2, Cat. no. N11999

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Contents

Chapter 1
Kit Content

Table 1-1. Thermo Scientific KingFisher Total RNA Kit

Item	KingFisher® Total RNA Kit, 1 x 96
Cat. No.	97020196
Package size	1 x 96 samples
KingFisher Magnetic Beads	3.1 ml
rDNase	3 vials
rDNase Buffer	35 ml
Reducing Agent TCEP	1 vial
Lysis Buffer	40 ml
Binding Buffer	75 ml
Wash Buffer 1	65 ml
Wash Buffer 2	200 ml
Elution Buffer	20 ml
RNase-free water	120 ml

The KingFisher Total RNA Kit (Cat. No. 97020196) is intended for the purification of cell or 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.

Equipment and reagents to be supplied by the user:

- Tween 20
- Magnetic particle processor
- Homogenization equipment (optional)

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 Total RNA Kit is designed for rapid automated purification of total RNA from cell and tissue samples using KingFisher instruments. The total RNA can be purified from up to 2 x 10^6 cells or 20 mg of tissue. The total RNA purified with the KingFisher Total RNA Kit is of high quality and free of proteins, nucleases, and other contaminants or inhibitors. Purified total RNA is suitable for direct use in many different downstream applications, such as PCR (polymerase chain reaction) after reverse transcription and in several other enzymatic reactions.

Intended use

The KingFisher Total RNA Kit is developed for the purification of total RNA from cell and tissue 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 Total RNA 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 Total RNA Kit for any particular use, because the performance of the kits has not been validated for any specific organism.

Principle and procedure

The KingFisher Total RNA Kit uses magnetic-particle technology for total RNA purification. The Thermo Scientific KingFisher technology combines the speed and efficiency of RNA purification with easy handling of magnetic particles. It is recommended that tissue

samples are mechanically disrupted in the Lysis Buffer before the purification can start to ensure a good yield of purified RNA. In case of cells, the sample should be pelleted before the addition of the Lysis Buffer. The samples are sedimented after a short centrifugation step and the cleared lysates are transferred to Thermo Scientific KingFisher plates for processing with a Thermo Scientific KingFisher magnetic particle processor. RNA binds to the surface of the Thermo Scientific KingFisher Magnetic Beads in the presence of a chaotropic salt. Co-purified DNA is removed during DNase treatment. The following effective wash steps dispose of proteins, cell debris and any residual contaminants, while the RNA bound to the KingFisher Magnetic Beads is transferred through the wash steps. Two different Wash Buffers are used, followed by a rapid rinse in 0.02% Tween 20 in RNase-free water or an air drying step, which considerably improves the purity of the total RNA. High-quality total RNA is eluted into the Elution Buffer and is ready for subsequent downstream processes, such as enzymatic reactions.

Kit specifications

The KingFisher Total RNA Kit is designed for rapid automated preparation of highly pure total RNA from cell and tissue samples using Thermo Scientific KingFisher magnetic particle processors. When excluding a dispense step requiring the addition of the Binding Buffer, the approximate processing time is 60 minutes for the purification of 96 samples in the KingFisher Flex or 12 samples in the KingFisher Duo and for the purification of 15 samples in the KingFisher mL. The obtained total RNA can be used directly in various downstream applications.

Up to 2 x 10^6 cells or 20 mg of tissue per sample can be used as sample material. The yields of acquired purified RNA depend on the sample type, the method of sample storage, and the method of tissue disruption. The KingFisher Total RNA Kit can be processed completely at room temperature.

The KingFisher Magnetic Beads are highly reactive, superparamagnetic beads. The binding capacity is approximately 1 µg of total RNA per 1 µl of KingFisher Magnetic 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 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 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 total RNA.

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 Total RNA 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 (max. 2 x 10^6 cells or 20 mg of tissue per sample with the KingFisher Total RNA Kit)	Up to 24 samples per run	Up to 12 samples per run	Up to 6 samples per run
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
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 2-2. Overview of Thermo Scientific KingFisher systems

	KingFisher mL	KingFisher
Processing volume	50–1000 µl	20–200 µl
Capacity	Up to 15 samples per run (max. 2 x 10^6 cells or 20 mg of tissue per sample with the KingFisher Total RNA 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 Total RNA Kit are available for the KingFisher Flex, the KingFisher Duo and the KingFisher mL. 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 Total RNA 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
Lysis Buffer	Guanidine thiocyanate < 60%	Harmful by inhalation, in contact with skin and if swallowed. Contact with acids liberates very toxic gas. Harmful for aquatic organisms, may cause long-term adverse effects in the aquatic environment. Keep away from food, drink and animal feed.
Binding Buffer	Isopropanol > 90%	Highly flammable. Irritating to eyes. Vapors may cause drowsiness and dizziness. Keep container tightly closed. Keep away from sources of ignition – no smoking. Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
Wash Buffer 1	Guanidine thiocyanate < 30% and ethanol < 45%	Flammable. Harmful by inhalation and in contact with skin. Keep away from food, drink and animal feed. Keep away from sources of ignition – no smoking.
Wash Buffer 2	Ethanol < 90%	Highly flammable. Keep container tightly closed. Keep away from sources of ignition – no smoking.
rDNase	Lyophilized rDNase	May cause sensitization by inhalation and skin contact. Do not inhale dust. Avoid contact with skin.
Reducing Agent TCEP	TCEP, Tris (2-carboxyethyl) phosphine hydrochloride	Irritating to eyes and 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 Solutions

Storage conditions

All buffers and reagents included in the KingFisher Total RNA 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 rDNase storage and working solutions

To prepare the rDNase storage solution for the KingFisher Total RNA Kit, add 800 µl of RNase-free water to each vial of the lyophilized rDNase and incubate at room temperature for 1 minute. Occasional gentle rotation of the vial enhances dissolvement of the rDNase, but avoid forceful mixing.

The rDNase storage solution should be stored at -20°C, where it remains stable for 6 months. Do not freeze and thaw the rDNase storage solution more than three times.

Calculate the amount of rDNase working solution needed. For the purification of one sample, mix 25 µl of rDNase storage solution and 275 µl of rDNase Buffer solution. The rDNase working solution should be used immediately after preparation.

Preparation of the Reducing Agent TCEP working solution

To prepare the Reducing Agent TCEP working solution for the KingFisher Total RNA Kit, add 750 µl of RNase-free water to a vial of Reducing Agent TCEP. Incubate the vial at room temperature for several minutes and mix occasionally to dissolve the Reducing Agent TCEP completely. The Reducing Agent TCEP working solution should be stored at -20°C.

Chapter 5
Protocols and Pipetting Instructions

Before beginning the total RNA 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.

Homogenization of samples

Use up to 2 x 10^6 of cultured cells or 20 mg of tissue per sample.

Efficient homogenization of the sample is an essential step before RNA purification in order to gain a good yield of high-quality RNA.

Tissue sample can be homogenized with various kinds of homogenizers, such as bead mills. The homogenization step must disrupt the structures of the sample material completely in order to ensure a high yield of RNA.

Homogenization of tissue samples

To homogenize *tissue samples*, add 350 µl of Lysis Buffer and 6 µl of Reducing Agent TCEP working solution to each sample and use a suitable homogenization instrument to disrupt the tissue. After the tissue has been homogenized, centrifuge the sample briefly (30 seconds, 1500 x g) and transfer the supernatant to a Thermo Scientific Microtiter deep well 96 plate or a Thermo Scientific KingFisher mL tube and begin the purification of RNA using the KingFisher Flex, the KingFisher Duo or the KingFisher mL. See the detailed instructions below.

Homogenization of cell samples

To homogenize *cell samples*, add 350 µl of Lysis Buffer and 6 µl of Reducing Agent TCEP working solution to a pelleted cell sample. Use a syringe or pipette to break the pellet by aspirating several times up and down and centrifuge briefly (30 seconds, 1500 x g). Transfer the supernatant to a Microtiter deep well 96 plate or a KingFisher mL tube and begin the purification of RNA using the KingFisher Flex, the KingFisher Duo or the KingFisher mL. See the detailed instructions below.

Instructions for KingFisher Flex with 96 deep well plates for total RNA purification from 350 µl of lysed cells or tissue

These instructions are for the total RNA purification from 350 µl of lysed cells or tissue using the KingFisher Total RNA Kit (Cat. No. 97020196) and the KingFisher Flex with Microtiter deep well 96 plates.

When using the KingFisher Total RNA Kit for the first time, prepare the storage and working solutions for rDNase and Reducing Agent TCEP. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Working Solutions".

1. Homogenize the samples according to the instructions given in "Homogenization of samples" on page 22.

2. Prepare the rDNase working solution for the samples that are used in a run and should be used immediately. First calculate the amount of rDNase working solution needed. For the purification of one sample, mix 25 µl of rDNase storage solution and 275 µl of rDNase Buffer solution.

3. Take six empty Microtiter deep well 96 plates and two empty Thermo Scientific KingFisher Flex 96 KF plates.

4. Prepare and fill six 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	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Homogenized sample	350 µl
			KingFisher Magnetic Beads	30 µl
			Binding Buffer	350 µl
2	Microtiter deep well 96 plate	DNase	rDNase working solution	300 µl
3	Microtiter deep well 96 plate	Wash 1	Wash Buffer 1	600 µl
4	Microtiter deep well 96 plate	Wash 2_1	Wash Buffer 2	900 µl
5	Microtiter deep well 96 plate	Wash 2_2	Wash Buffer 2	900 µl
6	Microtiter deep well 96 plate	Wash 3	0.02% Tween 20 in RNase-free water	1000 µl
7	KingFisher Flex 96 KF plate	Elution	Elution Buffer	150 µl

5. 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).

6. Start the KF_TotRNA_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_TotRNA_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_TotRNA_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*.

7. Add the Binding Buffer to the DNase plate during the dispense step.

When the KingFisher Flex pauses at the dispense step after the DNase digestion step (approximately 25 minutes after starting the run), remove the DNase plate from the instrument and separately add 350 µl of Binding Buffer per well to the DNase plate to rebind the RNA.

Plate number	Plate type	Plate name	Content	Reagent volume per well
2	Microtiter deep well 96 plate	DNase	Binding Buffer	350 µl

8. Place the DNase plate back into the instrument and press **Start**. After the pause, the protocol will continue to the end.

9. After the run is completed, remove the plates and store the purified total RNA.

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 total RNA accordingly. The purified total RNA is ready for use in downstream applications.

The final RNA concentration in the Elution Buffer may increase if the purified RNA is eluted into a smaller volume of the buffer than is recommended, but this can slightly reduce the overall RNA 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	Homogenized sample	350 µl
			KingFisher Magnetic Beads	30 µl
			Binding Buffer	350 µl
2	Microtiter deep well 96 plate	DNase	rDNase working solution	300 µl
Dispense step: add 350 µl of Binding Buffer per well to the DNase plate (Plate 2) during the pause in the BindIt protocol.				
3	Microtiter deep well 96 plate	Wash 1	Wash Buffer 1	600 µl
4	Microtiter deep well 96 plate	Wash 2_1	Wash Buffer 2	900 µl
5	Microtiter deep well 96 plate	Wash 2_2	Wash Buffer 2	900 µl
6	Microtiter deep well 96 plate	Wash 3	0.02% Tween 20 in RNase-free water	1000 µl
7	KingFisher Flex 96 KF plate	Elution	Elution Buffer	150 µl
8	KingFisher Flex 96 KF plate	Tip Plate		

Instructions for KingFisher Duo with 12-pin magnet head and 96 deep well plates for RNA purification of 350 µl lysed cell or tissue samples

These instructions are for the RNA purification from 350 µl of lysed cell or tissue samples using the KingFisher Total RNA Kit (Cat. No. 97020196) and the KingFisher Duo with a 12-pin magnet head and Microtiter deep well 96 plates.

When using the KingFisher Total RNA Kit for the first time, prepare the storage and working solutions for rDNase and Reducing Agent TCEP. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Working Solutions".

1. Homogenize the samples according to the instructions given in "Homogenization of samples" on page 22.

2. Prepare the rDNase working solution for the samples that are used in a run. The working solution should be used immediately. First calculate the amount of rDNase working solution needed. For the purification of one sample, mix 25 µl of rDNase storage solution and 275 µl of rDNase Buffer solution.

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

4. Prepare the **Total RNA 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 row C is 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
Total RNA plate Microtiter deep well 96 plate	A	Sample	Homogenized sample	350 µl
			KingFisher Magnetic Beads	30 µl
			Binding Buffer	350 µl
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	DNase	rDNase working solution	300 µl
	E	Wash 1	Wash Buffer 1	600 µl
	F	Wash 2_1	Wash Buffer 2	900 µl
	G	Wash 2_2	Wash Buffer 2	900 µl
	H	Wash 3	0.02% Tween 20 in RNase free water	1000 µl

5. 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

6. Place a Thermo Scientific KingFisher Duo 12-tip comb into **row B** on a **Total RNA plate**.

7. Start the KF_totRNA_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_totRNA_Duo protocol. Insert the Total RNA 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_totRNA_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*.

8. Add the Binding Buffer to row D during the dispense step.

When the KingFisher Duo pauses at the dispense step after the DNase digestion step (approximately 25 minutes after starting the run), remove the DNase plate from the instrument and separately add 350 µl of Binding Buffer per well to row D to rebind the RNA.

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Total RNA plate Microtiter deep well 96 plate	D	DNase	Binding Buffer	350 µl

9. Place the Total RNA plate back into the instrument and press **OK**. After the pause, the protocol will continue to the end.

10. After the run is completed, remove the plate and store the purified RNA.

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 RNA accordingly. The purified RNA is ready for use in downstream applications. The final RNA concentration in the Elution Buffer may increase if

the purified RNA is eluted into a smaller volume of the buffer than is recommended, but this can slightly reduce the overall RNA 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
Total RNA plate Microtiter deep well 96 plate	A	Sample	Homogenized sample	350 µl
			KingFisher Magnetic Beads	30 µl
			Binding Buffer	350 µl
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	DNase	rDNase working solution	300 µl
	Dispense step: add 350 µl of Binding Buffer per well to row D during the pause in the BindIt protocol.			
	E	Wash 1	Wash Buffer 1	600 µl
	F	Wash 2_1	Wash Buffer 2	900 µl
	G	Wash 2_2	Wash Buffer 2	900 µl
	H	Wash 3	0.02% Tween 20 in RNase free water	1000 µl
Elution strip		Elution	Elution Buffer	100 µl

Instructions for KingFisher mL for total RNA purification from 350 µl of lysed cells or tissue

These instructions are for the total RNA purification from 350 µl of lysed cells or tissue using the KingFisher Total RNA Kit (Cat. No. 97020196) and the KingFisher mL.

When using the KingFisher Total RNA Kit for the first time, prepare the storage and working solutions for rDNase and Reducing Agent TCEP. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Working Solutions".

A tube strip tray in the KingFisher mL may contain up to 15 separate Thermo Scientific KingFisher 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. Homogenize the samples according to the instructions given in "Homogenization of samples" on page 22.

2. Prepare the rDNase working solution for the samples that are used in a run and should be used immediately. First calculate the amount of rDNase working solution needed. For the purification of one sample, mix 25 µl of rDNase storage solution and 275 µl of rDNase Buffer solution.

3. Place empty KingFisher mL tubes 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	Sample/reagent volume
A	Sample	Homogenized sample	350 µl
		KingFisher Magnetic Beads	30 µl
		Binding Buffer	350 µl
B	DNase	rDNase working solution	300 µl
C	Wash1	Wash Buffer 1	600 µl
D	Wash2	Wash Buffer 2	900 µl
E	Elution	Elution Buffer	150 µl

4. 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.

5. Start the KF_TotRNA_mL protocol with the KingFisher mL.

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

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

6. Add the Binding Buffer to the DNase tubes during the dispense step.

When the KingFisher mL pauses at the dispense step after the DNase digestion step (approximately 25 minutes after starting the run), remove the tube strip tray from the instrument and separately add 350 µl of Binding Buffer to each DNase tube to rebind the RNA.

Tube	Tube name	Content	Reagent volume
B	DNase	Binding Buffer	350 µl

7. Place the tube strip tray 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 tube strips and store the purified total RNA.

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

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

Summary of tube contents

Table 5-3. Summary of tube contents

Tube	Tube name	Content	Sample/reagent volume
A	Sample	Homogenized sample	350 µl
		KingFisher Magnetic Beads	30 µl
		Binding Buffer	350 µl
B	DNase	rDNase working solution	300 µl
Dispense step: add 350 µl of Binding Buffer to each of the DNase tubes (B tubes) during the pause in the BindIt protocol.			
C	Wash1	Wash Buffer 1	600 µl
D	Wash2	Wash Buffer 2	900 µl
E	Elution	Elution Buffer	150 µl

Quantification and determination of the purity of RNA

It is recommended to measure the absorbance at 320 nm, 280 nm, and 260 nm. The concentration of RNA can be defined with the absorbance at 260 nm (A_{260}). One unit at 260 nm corresponds to 40 µg of RNA per ml. The ratio between the A_{260}/A_{280} indicates the purity of the RNA, and the value for RNA should be ≥ 1.8–2.1. A Thermo Scientific NanoDrop can be used for the direct measurement of RNA 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 magnetic beads.

Use these calculations to measure the RNA content:

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

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 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 RNA.

Binding and wash steps

A detergent reagent (Tween 20) is used in the wash 3 step. Avoid vigorous shaking of the bottle, as this will cause foam to form on the surface of the reagent, leading to problems while transferring the correct amount of the buffer to the respective wells or tubes.

The binding between the RNA and the KingFisher Magnetic Beads is strong in the presence of a chaotropic salt, but chaotropic salts are not present in the wash 3 step and accordingly the binding is weak. Avoid strong mixing speeds and releasing the beads into the wash 3 step in order to minimize the loss of RNA 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 RNA.

The volume of the Elution Buffer can be modified depending on the user requirements concerning the purified RNA concentration. The final RNA concentration in the Elution Buffer may increase if the purified RNA is eluted into a smaller volume of the buffer, but this can slightly reduce the overall RNA yield. The modifications of the volumes in 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 total RNA, 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 RNA from the beads.

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.

Appendix A
Troubleshooting

Table A-1. Troubleshooting guide

Problem	Possible cause and actions
Low total RNA 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. Efficient lysis of the cells or tissue increases the total RNA yield. Prolonged storage of the sample material may reduce the total RNA yield. If the mixing is too strong, it may cause partial elution of total RNA already during the wash 3 step. 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 total RNA.
Low purity	Prolonged storage of the sample material may reduce the quality and quantity of the total RNA. Insufficient washing causes impurities in the Elution Buffer. The Wash Buffers 1 and 2 contain 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 affect 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 41. KingFisher Magnetic Beads that occasionally remain attached to the tip combs at the end of the process do not affect the total RNA yield, as the RNA 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 Total RNA Kits

Cat. No.	Product	Package size
97020196	KingFisher Total RNA 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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