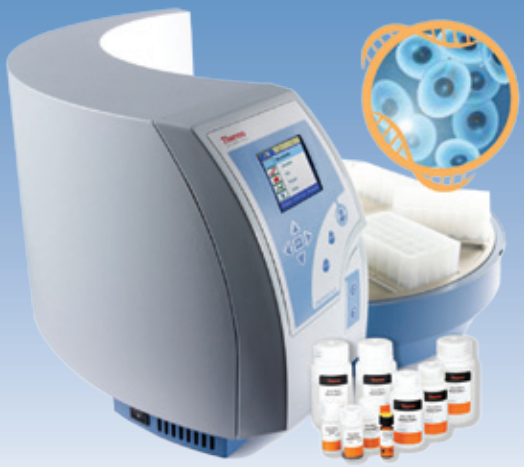

Instruction Manual

Thermo Scientific KingFisher Pure RNA Tissue Kit

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

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Kit Content

Table 1-1. Thermo Scientific™ KingFisher™ Pure RNA Tissue Kit

Item	KingFisher Pure RNA Tissue Kit	
Cat. No.	98040196	98040496
Package size	96 samples	384 samples
Lysis Buffer	50 ml	200 ml
KingFisher Magnetic Beads	3 x 1.4 ml	2 x 8.5 ml
DNase I (lyophilized)	1 vial	1 vial
DNase I Reconstitution Buffer	1 ml	2.5 ml
10 x Reaction Buffer with $MgCl_2$ for DNase I	3 x 1 ml	9 x 1 ml
Wash Buffer 1 (conc.)*	50 ml	2 x 100 ml
Wash Buffer 2 (conc.)*	45 ml	3 x 45 ml
Nuclease-free water	30 ml	125 ml

* Addition of ethanol required.

The KingFisher Pure RNA Tissue Kit (Cat. No. 98040196 or 98040496) is intended for the purification of RNA, using the Thermo Scientific™ KingFisher™ Flex with a 96 deep well head or the Thermo Scientific™ KingFisher™ Duo with a 12-pin head from up to 2 x 10^6 cells or up to 20 mg tissue samples.

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

Storage Conditions

Upon arrival of the kit, store the DNase I, DNase I Reconstitution Buffer, and 10 x Reaction Buffer with $MgCl_2$ for DNase I at -20°C. The reconstituted DNase I should be stored at -20°C. Store the Thermo Scientific™ KingFisher™ Magnetic Beads at +4°C. Other kit components can be stored at room temperature (15–25°C). The reagents are stable for up to two years from the manufacturing date.

Additional Reagents Required

- 96–100% ethanol (EtOH), molecular biology grade
- 2 M DTT (dithiothreitol) or 14.3 M ß-mercaptoethanol solution
- 1 x PBS for mammalian cell cultures

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

Cat. No.	Product
5400100	KingFisher Duo magnetic particle processor
5400630	KingFisher Flex magnetic particle processor with 96 deep well head

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

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

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

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

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Product Description

Introduction

The KingFisher Pure RNA Tissue Kit is designed for rapid automated purification of RNA from up to 2 x 10^6 cells or up to 20 mg tissue samples using Thermo Scientific™ KingFisher™ instruments. The RNA purified using the KingFisher Pure RNA Tissue Kit is of high quality and free of proteins, genomic DNA, nucleases, and other contaminants or inhibitors. It is, therefore, suitable for direct use in many different downstream applications, such as RT-qPCR (reverse transcription quantitative PCR), RT-PCR, and several other enzymatic reactions.

Intended Use

The KingFisher Pure RNA Tissue Kit is developed for purification of RNA from tissue samples using paramagnetic particles. The reagents and specific plastic consumables are designed for use with the KingFisher Flex and KingFisher Duo magnetic particle processors as part of an integrated system. The KingFisher Pure RNA Tissue Kit is only intended for research use, not for clinical or diagnostic use. The user is responsible for validating the performance of the KingFisher instrument and the KingFisher Pure RNA Tissue Kit for any particular use, as the performance of the kits has not been validated for any specific organism or downstream application.

Principle and Procedure

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

It is recommended to mechanically disrupt tissue samples in Lysis Buffer. In case of cells, the sample should be pelleted before addition of the Lysis Buffer. The first step of the protocol lyses the cells, after which the NA (nucleic acids) can bind to the surface of the KingFisher Magnetic Beads in the presence of a chaotropic salt. Copurified DNA is removed during a DNase I treatment step.

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 an air drying step. High-quality RNA is eluted into nuclease-free water, and is ready for subsequent downstream processes.

Kit Specifications

The KingFisher Pure RNA Tissue Kit is designed for rapid automated preparation of highly pure total RNA from up to 2 x 10^6 cells or up to 20 mg tissue samples using KingFisher magnetic particle processors. If a dispense step requiring the addition of ethanol is excluded, the approximate processing time is 60 minutes for the purification of 96 samples on the KingFisher Flex and 12 samples on the KingFisher Duo. The obtained RNA can be used directly in various downstream applications.

Fresh or frozen cells or tissue samples can be used. The procedure is optimized for up to 2 x 10^6 cells or up to 20 mg tissue samples in 450 µl of Lysis Buffer. Suitable sample storage and an efficient homogenization step are essential for obtaining a high yield and good quality total RNA.

Depending on the sample, typically 20–150 µg of total RNA can be purified from 20 mg of fresh tissue sample with an A_{260}/A_{280} ratio of ≥ 1.8–2.1. The yields of acquired purified RNA depend on the sample type, and the method of sample collection, storage, and disruption. Refer to Appendix B for typical total RNA yields from various sources.

KingFisher Magnetic Particle Processors

The KingFisher magnetic particle processors are designed for the automated transfer and processing of magnetic particles in microplate format. The patented technology of the Thermo Scientific™ KingFisher™ systems is based on the use of magnetic rods covered with a disposable, specially

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

The KingFisher instrument family comprises four systems covering working volumes from 20 to 5000 µl. Each system consists of an instrument, specially designed plastic consumables, and the easy-to-use BindIt Software 3.2. The KingFisher Pure RNA Tissue Kit is optimized and ready for use with the KingFisher Flex or KingFisher Duo.

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

The BindIt Software 3.2 protocols optimized for the KingFisher Pure RNA Tissue Kit are available for the KingFisher Flex and KingFisher Duo. For more information, go to www.thermoscientific.com/kingfisherinfo or contact your local authorized distributor.

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

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

* See the details above on the Plates row.

3

Safety Information

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

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

Table 3-1. Safety precautions

Reagent	Hazardous contents	Safety instructions
Lysis Buffer	Guanidium thiocyanate	Harmful by inhalation. Liberates very toxic gas in contact with acids. Harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment. Keep the container in a well-ventilated place. Do not breathe gas/fumes/vapor/spray. Wear suitable protective clothing and gloves. This material and its container must be disposed of as hazardous waste. Avoid release to the environment. Refer to special instructions/safety data sheets.

Continued

Cont.

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

4

Storage Conditions and Preparation of the Reagents

Storage Conditions

Upon arrival of the kit, store the DNase I, 10 x Reaction Buffer with $MgCl_2$ for DNase I, and DNase I Reconstitution Buffer at -20°C. The reconstituted DNase I should be stored at -20°C. Store the KingFisher Magnetic Beads at +4°C. Other kit components can be stored at room temperature (15–25°C). The reagents are stable for up to two years from the manufacturing date.

Preparation of the Lysis Buffer

Before each RNA purification, a fresh aliquot of Lysis Buffer should be supplemented with DTT or ß-mercaptoethanol solutions (not provided). Calculate the amount of Lysis Buffer needed. For purification of one sample, 450 µl of Lysis Buffer is required. Add 10 µl of 2 M DTT or 10 µl of 14.3 M ß-mercaptoethanol to each 450 µl of Lysis Buffer.

Preparation of the Wash Buffers

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

Table 4-1. Instructions for the preparation of Wash Buffer 1 and Wash Buffer 2. Add the indicated volume of 96–100% ethanol to each bottle.

	96 samples (Cat. No. 98040196)		384 samples (Cat. No. 98040496)	
	Wash Buffer 1	Wash Buffer 2	Wash Buffer 1	Wash Buffer 2
Concentrated buffer	50 ml	45 ml	100 ml	45 ml
Ethanol (96–100%)	50 ml	180 ml	100 ml	180 ml
Total volume	100 ml	225 ml	200 ml	225 ml

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

Preparation of the DNase I Storage Solution

To prepare the DNase I storage solution, add 550 µl (96 samples kit) or 2.2 ml (384 samples kit) of DNase I Reconstitution Buffer to each vial of the lyophilized DNase I. Incubate at room temperature for 5 min. Occasional gentle rotation of the vial helps to dissolve the DNase I, but avoid forceful mixing. Store the DNase I storage solution at -20°C. Repeated freezing and thawing should be avoided.

Preparation of the DNase I Reaction Buffer

Before each RNA purification, calculate the amount of 1 x DNase I Reaction Buffer needed. For purification of one sample, mix 20 µl of 10 x Reaction Buffer with $MgCl_2$ for DNase I and 180 µl of nuclease-free water. This buffer should be used immediately after preparation.

5

Protocols and Pipetting Instructions

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

BindIt Software 3.2 protocols for the KingFisher Pure RNA Tissue Kit can be found in BindIt Software 3.2 and at www.thermoscientific.com/kingfisher.

Handling of KingFisher Magnetic Beads

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

Avoiding Ribonuclease (RNase) Contamination

RNA purity and integrity is essential for downstream applications. RNase is a ubiquitously found enzyme, which degrades RNA. RNases are highly stable contaminants found in any laboratory environment. Keep all kit components tightly sealed when not in use.

Skin is a common source of RNases. Always wear gloves when handling reagents and RNA samples. Use sterile, RNase-free pipette tips when working with RNA. Remove RNase contamination from work surfaces and non-disposable items (pipettes, centrifuges) with reagents designed to remove RNase.

Homogenization of Sample Material

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

Homogenization of Cell Samples

Collect up to 2 x 10^6 of *cultured cells* into a centrifuge tube. Pellet the cells by centrifugation for 5 min at 3,000 x g. Discard the supernatant. Rinse the cells once with 1 x PBS to remove residual medium and repeat the centrifugation step. Discard the supernatant. Resuspend the collected cells in 450 µl of Lysis Buffer containing either DTT or ß-mercaptoethanol. Mix by vortexing to obtain a uniform suspension. Transfer the prepared lysate to a Thermo Scientific™ Microtiter™ deep well 96 plate and begin the purification using the KingFisher Flex or KingFisher Duo. Refer to the detailed instructions below.

Homogenization of Tissue Samples

Grind up to 20 mg of mammalian *tissue* in liquid nitrogen using a mortar and pestle or in an available homogenizator. Add 450 µl of Lysis Buffer, containing either DTT or ß-mercaptoethanol, to the tissue sample before or after homogenization, depending on the system. Mix by vortexing to obtain a uniform suspension. Spin down the tube to collect all the drops from the walls of the tube. Transfer the prepared lysate to a Microtiter deep well 96 plate and begin the purification using the KingFisher Flex or KingFisher Duo. Refer to the detailed instructions below.

Instructions for KingFisher Flex with 96 Deep Well Plates

These instructions are intended for total RNA purification from 450 µl of lysate, using the KingFisher Pure RNA Tissue Kit (Cat. No. 98040196 or 98040496) and the KingFisher Flex with 96 deep well plates.

When using the KingFisher Pure RNA Tissue Kit for the first time, dissolve the DNase I in the DNase I Reconstitution Buffer. Then prepare the Wash Buffer 1 and Wash Buffer 2. Before every purification, prepare the 1 x

DNase I Reaction Buffer and add DTT or ß-mercaptoethanol to the Lysis Buffer. For detailed instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Reagents".

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).

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

2. Fill the **plates** as follows.

Plate number	Plate type	Plate name	Content	Reagent volume per well
1	Microtiter deep well 96 plate	Sample	Lysed sample KingFisher Magnetic Beads* Ethanol	450 µl 40 µl 400 µl
2		DNase	1 x DNase I Reaction Buffer DNase I (storage solution)**	200 µl 5 µl
3		Wash 1	Wash Buffer 1	900 µl
4		Wash 2_1	Wash Buffer 2	700 µl
5		Wash 2_2	Wash Buffer 2	700 µl
6	KingFisher Flex 96 KF plate	Elution	Nuclease-free water	100 µl

* Resuspend the KingFisher Magnetic Beads well by vortexing before use.
** Avoid long-term storage of DNase I in 1 x DNase I Reaction Buffer solution. It is recommended to add the DNase I solution to the plate as the last step.

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

4. Start the PURE_RNATissue_Flex96 protocol using the KingFisher Flex 96 and load the plates as instructed on the KingFisher Flex 96 instrument display.

Switch on the KingFisher Flex making sure that you are using the Thermo Scientific™ KingFisher™ Flex 96 deep well head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Flex. Start the PURE_RNATissue_Flex96 protocol. Insert the Tip Plate and the filled plates into the instrument as indicated on the KingFisher Flex display. After all the plates have been loaded into the instrument, the protocol will start.

When the KingFisher Flex is to be run as a standalone instrument, transfer the PURE_RNATissue_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*.

5. Add ethanol to the DNase plate during the dispense step.

When the KingFisher Flex pauses at the dispense step after the DNase digestion step at approximately 25 minutes after starting the protocol run, remove the DNase plate from the instrument. Then add 200 µl of ethanol to each well to rebind the RNA.

Plate number	Plate type	Plate name	Content	Reagent volume per well
2	Microtiter deep well 96 plate	DNase	Ethanol	200 µl

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

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

When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and switch off the instrument. The purified RNA is ready for use in downstream applications. When working with RNA, keep the purified samples on ice. Store the purified RNA at -20°C or -80°C.

NOTE: The final RNA concentration in the nuclease-free water may increase if the purified RNA is eluted into a smaller than recommended volume of water, but this can slightly reduce the overall RNA yield.

Instructions for KingFisher Duo with 12-pin Magnet Head

These instructions are intended for RNA purification from 450 µl of lysate, using the KingFisher Pure RNA Tissue Kit (Cat. No. 98040196 or 98040496) and the KingFisher Duo with 12-pin magnet head.

When using the KingFisher Pure RNA Tissue Kit for the first time, dissolve the DNase I in the DNase I Reconstitution Buffer. Then prepare the Wash Buffer 1 and Wash Buffer 2. Before every purification, prepare 1 x DNase I Reaction Buffer and add DTT or ß-mercaptoethanol to the Lysis Buffer. For detailed instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Reagents".

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).

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

2. Prepare the **Tissue RNA plate** (i.e. a Microtiter deep well 96 plate).

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

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Tissue RNA plate Microtiter deep well 96 plate	A	DNase	1 x DNase I Reaction Buffer DNase I (reconstituted)*	200 µl 5 µl
	B	Tip	12-tip comb	Empty
	C	Sample	Lysed sample KingFisher Magnetic Beads** Ethanol	450 µl 40 µl 400 µl
	D	Empty	Empty	Empty
	E	Empty	Empty	Empty
	F	Wash 1	Wash Buffer 1	900 µl
	G	Wash 2_1	Wash Buffer 2	700 µl
	H	Wash 2_2	Wash Buffer 2	700 µl

* Avoid long-term storage of DNase I in 1 x DNase I Reaction Buffer at room temperature. It is recommended to add the DNase I solution to the plate as the last step.

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

3. Fill the KingFisher Duo elution strip as follows. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and the nuclease-free water is pipetted into the correct wells.

Elution strip	Content	Reagent volume per well
KingFisher Duo elution strip	Nuclease-free water	100 µl

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

5. Start the PURE_RNATissue_Duo protocol using the KingFisher Duo and load the plate and elution strip.

Switch on the KingFisher Duo making sure that you are using the Thermo Scientific™ KingFisher™ Duo 12-pin magnet head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Duo. Start the PURE_RNATissue_Duo protocol. Insert the Tissue 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 PURE_RNATissue_Duo protocol to the KingFisher Duo. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add ethanol to row A during the dispense step.

When the KingFisher Duo pauses at the dispense step after the DNase digestion step at approximately 25 minutes after starting the protocol run, remove the **Tissue RNA plate** from the instrument. Then add 200 µl of ethanol to each well in **row A** to rebind the RNA.

Row	Row name	Content	Reagent volume per well
A	DNase	Ethanol	200 µl

7. Place the Tissue RNA plate back into the instrument and press **OK**. After the pause, the protocol will continue to completion.

8. After the run is completed, remove the plate and elution strip, and store the purified RNA.

When the protocol is completed, remove the plates according to the instructions on the KingFisher Duo display and switch off the instrument. The purified RNA is ready for use in downstream applications. When working with RNA, keep the purified samples on ice. Store the purified RNA at -20°C or -80°C.

NOTE: The final RNA concentration in the nuclease-free water may increase if the purified RNA is eluted into a smaller than recommended volume of water, but this can slightly reduce the overall RNA yield.

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. The value for RNA should be ≥ 1.8–2.1.

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

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

6

General Information

Reagent Specificity and Volumes

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

Handling of Magnetic Beads

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

Binding, Wash, and Elution Steps

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

The volume of nuclease-free water used for elution can be modified depending on user requirements concerning the purified RNA concentration. The final RNA concentration may increase if the purified RNA is eluted into a smaller than recommended volume of water, but this can slightly reduce the overall RNA yield. The modifications of the elution step must be done in BindIt Software 3.2 and according to the volume ranges suitable for the KingFisher instrument. The table below indicates the available elution volumes of the KingFisher instruments.

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

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

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

Decontamination and Disinfection of Sample Material

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

A

Troubleshooting

Problem	Possible cause and actions
Low RNA yield	There should be an adequate volume of water to completely cover the KingFisher Magnetic Beads during the elution step. Do not let the KingFisher Magnetic Beads dry as this may result in lower elution efficiency. Efficient homogenization of the samples increases the total RNA yield. Prolonged storage of the sample material may reduce the total RNA yield. Use only Thermo Scientific plates, strips, and tip combs with the KingFisher instruments. Use of products from other manufacturers may cause unsuitable mixing and affect the yield of purified 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 eluted RNA.

Continued

Cont.

Problem	Possible cause and actions
Magnetic particles remaining in the sample or elution well	Starting material that is too viscose prevents efficient collection of the KingFisher Magnetic Beads from the lysed sample. The magnetic rods will not be able to collect all the particles unless the viscose samples are diluted before the beginning of the purification process. Improper lysis may also cause problems collecting the KingFisher Magnetic Beads. If the KingFisher Magnetic Beads are inefficiently collected from the elution step, the addition of a small amount of detergent (e.g. Tween™ 20) may improve the results. KingFisher Magnetic Beads that occasionally remain attached to the tip combs at the end of the process do not affect the 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.

B

Typical RNA Yields

Table B-1. Typical total RNA yields from various sources using KingFisher magnetic particle processors

Source	Quantity of starting material	RNA yield (µg)
Jurkat	10^6 cells	10–11
HeLa	10^6 cells	20–30
COS-7	5×10^5 cells	15–17
Mouse heart	5 mg	4–5
Mouse heart	20 mg	18–20
Mouse liver	5 mg	37–45
Mouse liver	20 mg	140–150
Mouse spleen	5 mg	27–30
Mouse brain	5 mg	4–7
Mouse lung	5 mg	5–9
Mouse kidney	5 mg	12–14
Mouse muscle	5 mg	3–4
E. coli	~10^9 cells	23–25
Saccharomyces cerevisiae	~10^8 cells	22–25

C

Ordering Information

Table C-1. KingFisher Pure RNA Tissue Kits

Cat. No.	Product	Package size
98040196	KingFisher Pure RNA Tissue Kit	96
98040496	KingFisher Pure RNA Tissue Kit	384

Table C-2. KingFisher Flex consumables

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

Table C-3. KingFisher Duo consumables

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

Notes

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