Instruction Manual

Thermo Scientific KingFisher Pure RNA Plant 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 Plant Kit

Item	KingFisher Pure RNA Plant Kit	
Cat. No.	98060196	98060496
Package size	96 samples	384 samples
Lysis Buffer	72 ml	2 x 140 ml
KingFisher Magnetic Beads	2 x 1.4 ml	10.6 ml
DNase I (lyophilized)	1 vial	4 vials
DNase I Reconstitution Buffer	1 ml	2 x 1 ml
2 x DNase I Buffer	12 ml	45 ml
Manganese Chloride Solution	3 x 1 ml	9 x 1 ml
Rebinding Buffer (conc.)*	20 ml	70 ml
Wash Buffer 1 (conc.)*	125 ml	3 x 125 ml
Wash Buffer 2 (conc.)*	50 ml	3 x 50 ml
Nuclease-free water	30 ml	125 ml

* Addition of ethanol required.

The KingFisher Pure RNA Plant Kit (Cat. No. 98060196 or 98060496) is intended for the purification of plant samples, using the Thermo Scientific™ KingFisher™ Flex with a 96 deep well head or the Thermo Scientific™ KingFisher™ Duo with a 12-pin head and a sample volume of 50 mg of fresh plant tissue.

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 Manganese Chloride Solution at -20°C. Also after dissolving of the DNase I, continue to keep it 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) solution
- Polyvinylpyrrolidone (PVP) for woody, lignified, or polyphenol-rich samples

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

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

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

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

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

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

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Product Description

Introduction

The KingFisher Pure RNA Plant Kit is designed for rapid automated purification of RNA from plant samples using Thermo Scientific™ KingFisher™ instruments. The RNA purified using the KingFisher Pure RNA Plant Kit is of high quality and free of proteins, nucleases, and other contaminants or inhibitors. It is, therefore, suitable for direct use in many different downstream applications, such as RT-qPCR (reverse transcription quantitative PCR), RT-PCR, and several other enzymatic reactions.

Intended Use

The KingFisher Pure RNA Plant Kit is developed for purification of total RNA from plant 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 Plant Kit enables extraction of RNA from fresh samples, or samples frozen immediately after collection and stored at -80°C. The KingFisher Pure RNA Plant Kit is only intended for research use, not for clinical or diagnostic use. The user is responsible for validating the performance of the Thermo Scientific™ KingFisher™ instrument and the KingFisher Pure RNA Plant 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 Plant 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. The purification process requires no phenol/chloroform extraction and needs very little hands-on time.

To ensure a good yield of purified RNA, the plant samples should be mechanically disrupted efficiently before beginning the purification. The homogenized samples are resuspended into the Lysis Buffer. Then the samples are incubated and sedimented using a short centrifugation. The cleared lysates are transferred to the Thermo Scientific™ KingFisher™ plates for processing with a KingFisher magnetic particle processor. The first step of the protocol further lyses the samples, after which the RNA can bind to the surface of the KingFisher Magnetic Beads in the presence of the Binding Buffer. The KingFisher Magnetic Beads are highly reactive, superparamagnetic beads. Copurified 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 an air drying step. High-quality RNA is eluted into the Elution Buffer, and is ready for subsequent downstream processes.

Kit Specifications

The KingFisher Pure RNA Plant Kit is designed for rapid automated preparation of highly pure total RNA from plant samples using KingFisher magnetic particle processors. If a dispense step requiring the addition of the Binding Buffer 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 plant samples can be used. A suitable amount of fresh material is 20–50 mg. Use of young plant samples, and/or if possible keeping plants for 12 h in darkness before collecting the samples, reduces the polysaccharide and polyphenolic contents, which may interfere in downstream applications. Suitable sample storage as well as an efficient homogenization step is essential for obtaining a high yield and good quality total RNA. Typically 5–50 µg of total RNA can be purified from 50 mg of fresh plant 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.

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 total 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 Plant Kit is optimized and ready for use with the KingFisher Flex or KingFisher Duo.

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.

The BindIt Software 3.2 protocols optimized for the KingFisher Pure RNA Plant 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 formats	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 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 Plant Kit contain hazardous contents (Table 3-1).

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

Table 3-1. Safety precautions

Reagent	Hazardous contents	Safety instructions
Lysis Buffer	Guanidium thiocyanate, Sodium N-layroylsarcosinate	Harmful by inhalation, in contact with skin and if swallowed. 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
Rebinding Buffer (conc.)	Guanidium 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.
Wash Buffer 1 (conc.)	Guanidium 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, DNase I Reconstitution Buffer, and Manganese Chloride Solution at -20°C. Also after dissolving of the DNase I, continue to keep it 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 (not provided). Calculate the amount of Lysis Buffer needed. It is required to use 600 µl of Lysis Buffer for the purification of one sample. Add 20 µl of 2 M DTT to each 1 ml 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. 98060196) and 384 samples (Cat. No. 98060496)	
	Wash Buffer 1	Wash Buffer 2
Concentrated buffer	125 ml	50 ml
Ethanol (96–100%)	125 ml	200 ml
Total volume	250 ml	250 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 440 µl 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 Working Solution

Before each RNA purification, calculate the amount of DNase I working solution needed. For the purification of one sample, mix 100 µl of 2 x DNase I Buffer, 20 µl of Manganese Chloride Solution, 4 µl of DNase I storage solution, and 76 µl of nuclease-free water. The DNase I working solution should be used immediately after preparation.

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

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

Homogenize 20–50 mg of fresh plant sample. Follow the instructions of the available homogenization tool. Add before or after homogenization, depending on the system, 600 µl of Lysis Buffer, including 2 M DTT to each sample. Incubate the sample at 56°C for 5 min. To clear the plant lysate, centrifuge the sample at 20,000 x g for 10 min. Transfer 400 µl of cleared supernatant 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.

Instructions for KingFisher Flex with 96 Deep Well Plates

These instructions are intended for RNA purification from 400 µl of plant lysate, using the KingFisher Pure RNA Plant Kit (Cat. No. 98060196 or 98060496) and the KingFisher Flex with 96 deep well plates.

When using the KingFisher Pure RNA Plant Kit for the first time, prepare the DNase I storage solution, Rebinding Buffer, Wash Buffer 1, and Wash Buffer 2. For each run, prepare the DNase I working solution and Lysis Buffer with 2 M DTT. For more 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 six 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	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Plant lysate KingFisher Magnetic Beads* Ethanol	400 µl 25 µl 400 µl
2		Wash 1_1	Wash Buffer 1	900 µl
3		DNase	DNase I working solution	200 µl
4		Wash 1_2	Wash Buffer 1	700 µl
5		Wash 2_1	Wash Buffer 2	700 µl
6		Wash 2_2	Wash Buffer 2	700 µl
7	KingFisher Flex 96 KF plate	Elution	Nuclease-free water	100 µl

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

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_RNAPlant_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_RNAPlant_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_RNAPlant_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 Rebinding Buffer and ethanol to the Sample plate during the dispense step.

When the KingFisher Flex pauses at the dispense step after the lysis step at approximately 25 minutes after starting the protocol run, remove the DNase plate from the instrument, and add the Rebinding Buffer and ethanol to the **DNase plate** to rebind the RNA. One can make a premix of Rebinding Buffer and ethanol if desired.

Plate name	Add	Added reagent volume per well
DNase	Rebinding Buffer	150 µl
	Ethanol	400 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 400 µl of plant lysate, using the KingFisher Pure RNA Plant Kit (Cat. No. 98060196 or 98060496) and the KingFisher Duo with 12-pin magnet head.

When using the KingFisher Pure RNA Plant Kit for the first time, prepare the DNase I storage solution, Rebinding Buffer, Wash Buffer 1, and Wash Buffer 2. For each run, prepare the DNase I working solution and Lysis Buffer with 2 M DTT. For more 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 **Plant 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 row C is also left *empty*.

Plate name and type	Row	Row name	Content	Sample/ reagent volume per well
Plant RNA plate Microtiter deep well 96 plate	A	DNase	DNase I working solution	200 µl
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	Wash 2_2	Wash Buffer 2	700 µl
	E	Wash 2_1	Wash Buffer 2	700 µl
	F	Wash 1_2	Wash Buffer 1	700 µl
	G	Wash 1_1	Wash Buffer 1	900 µl
	H	Sample	Plant lysate KingFisher Magnetic Beads*	400 µl 25 µl
			Ethanol	400 µl

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

3. Fill the KingFisher Duo elution strip as follows. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and the 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 **Plant RNA plate**.

5. Start the PURE_RNAPlant_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_RNAPlant_Duo protocol. Insert the Plant 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_RNAPlant_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 Rebinding Buffer and ethanol to row A during the dispense step.

When the KingFisher Duo pauses at the dispense step after the lysis step at approximately 25 minutes after starting the protocol run, remove the plate from the instrument, and add the Rebinding Buffer and ethanol into **row A** on the **Plant RNA plate** to rebind the RNA. One can make a premix of Rebinding Buffer and ethanol if desired.

Row	Row name	Add	Added reagent volume per well
A	DNase	Rebinding Buffer Ethanol	150 µl 400 µl

7. Place the 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 plate and elution strip 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 total 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 the Elution Buffer can be modified depending on user requirements concerning the purified total RNA concentration. The final RNA concentration in the Elution Buffer may increase if the purified RNA is eluted into a smaller than recommended volume of the buffer, 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 volumes of Elution Buffer in the KingFisher instruments. The Elution Buffer should cover the KingFisher Magnetic Beads completely, and any possible sedimented magnetic-bead pellet(s) 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.

A

Troubleshooting

Problem	Possible cause and actions
Low 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 homogenization of the plant 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 Buffer, 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

Ordering Information

Table B-1. KingFisher Pure RNA Plant Kits

Cat. No.	Product	Package size
98060196	KingFisher Pure RNA Plant Kit	96
98060496	KingFisher Pure RNA Plant Kit	384

Table B-2. KingFisher Flex consumables

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

Table B-3. KingFisher Duo consumables

Cat. No.	Product	
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

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