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Total RNA Purification Kit (Magnetic Bead System) – Supplementary Protocol

Product # 75400 & 75500

Using KingFisher Flex with 96 Deep-Well Magnetic Head

Component Required

Component (Either One)	Product Number
Total RNA Purification Kit (Magnetic Bead System)	75400
Total RNA Purification Kit 96-Well Kit (Magnetic Bead System)	75500

Customer-Supplied Reagents and Equipment

- Micropipettors
- Microcentrifuge tubes
- β-mercaptoethanol (optional)
- Isopropanol
- Temperature adjustable (37ºC) incubator - (for Yeast protocol)
- Nuclease-free water
- DNase I (optional)
- Phosphate Buffered Saline (PBS) – (for Cultured Animal Cells protocol)
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer - (for Animal Tissue protocol)
- Syringe with a 22G needle - (for Animal Tissue protocol)
- KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head (Cat. 5400630)
- KingFisher™ Flex 96 Deep-Well heating block (24075430)
- KingFisher™ Deep-Well 96 plate (95040450 or 95040460)
- KingFisher™ 96 tip comb for Deep-Well magnets (97002534)
- KingFisher™ Flex 96 KF heating block (24075420) – *optional*
- KingFisher™ 96 KF plate (97002540) – *optional*
- Follow the requirements for each of the sample types as mentioned in the product inserts of kits 75400 & 75500

Notes prior to use:

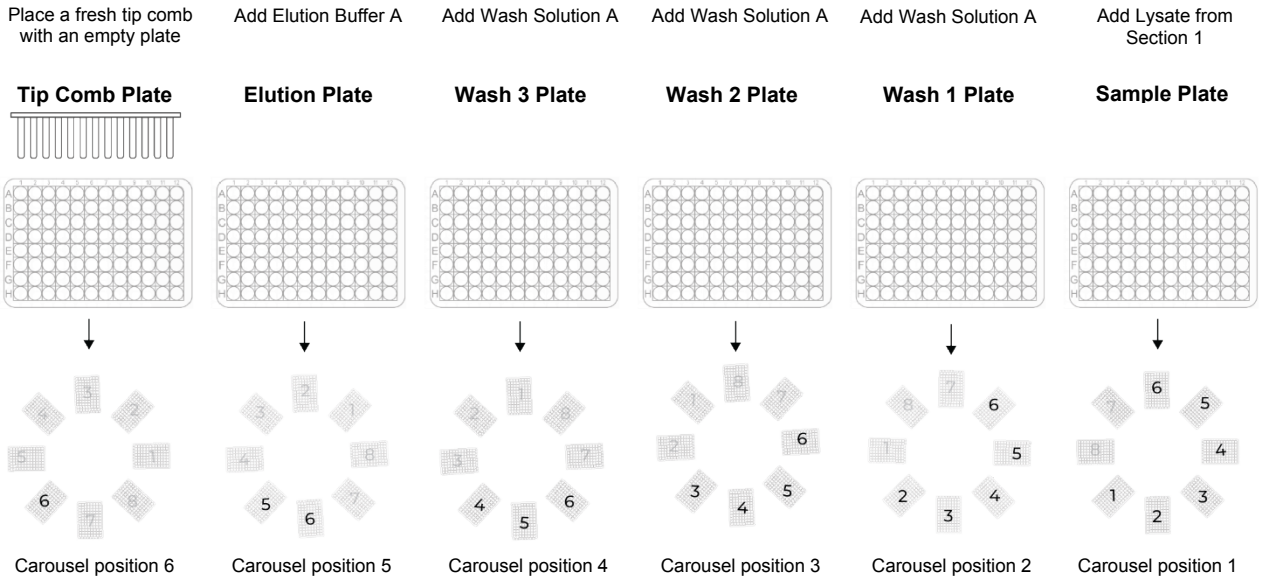
- The procedure provides information and steps to follow to extract RNA using automated methods using KingFisher™ Flex platform.
- Please follow all the instructions mentioned in the product insert of the kits 75400 or 75500.
- The isolation can be performed by using the following combination of heating blocks and plates

Instrument	Heat Block	Sample and Wash Plate type	Elution Plate type
KingFisher Flex	96 Deep-Well heating block (24075430) *	Deep-Well 96 plate (95040450 or 95040460)	Deep-Well 96 plate (95040450 or 95040460)
	96 KF heating block (24075420)		96 KF plate (97002540)

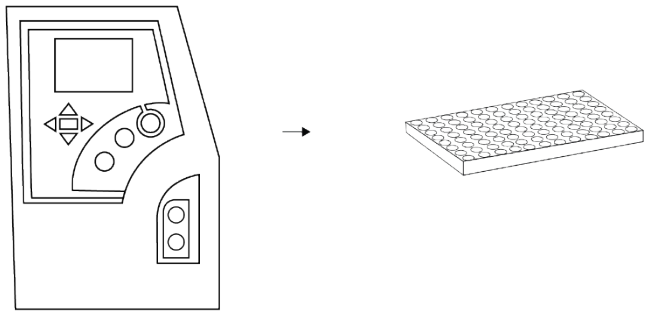
**96 Deep-Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution step requires a heating step. Using incompatible plates can lead to reduced RNA yield.*

Flow Chart

Procedure for using Norgen's Total RNA Purification Kit (Magnetic Bead System) – KingFisher™ Flex 96



Run Instrument



Place Sample Plate at position 1 and continue to run program.

Run Complete. Take Elution plate and store RNA.

Table 1. Set-up for RNA Extraction

Prepare plates as per the volumes mentioned in the table below. Detailed description of step-wise process can be found in **Section 1 and 2.**

Table 1a. Setup of plates while using KingFisher™ Flex with a 96 Deep-Well Head and a 96 Deep-Well heating block.

Set-up while using 96 Deep-Well heating block with 96 Deep-Well Head*				
Component	**Plate ID**	**Location on Carousel**	**Plate Type**	**Amount**
Lysate	Sample	1	deep-well	Variable
Wash Solution A	Wash 1	2	deep-well	600 µL
Wash Solution A	Wash 2	3	deep-well	600 µL
Wash Solution A	Wash 3	4	deep-well	600 µL
Elution Buffer A	Elution	5	deep-well	100 µL
Tip Comb	Tip Comb	6	Place a 96 Deep-Well Tip Comb in a Deep-Well plate	

**Refer to the table (1a or 1b) based on the heating block and plates being used. 96 Deep-Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution requires a heating step. Using incompatible plates can lead to reduced RNA yield.*

Table 1b. Setup of plates while using KingFisher™ Flex with a 96 Deep-Well Head and a 96 KF heating block.

Set-up while using 96 heating KF block with 96 Deep-Well Head*				
Component	**Plate ID**	**Location on Carousel**	**Plate Type**	**Amount**
Lysate	Sample	1	deep-well	Variable
Wash Solution A	Wash 1	2	deep-well	600 µL
Wash Solution A	Wash 2	3	deep-well	600 µL
Wash Solution A	Wash 3	4	deep-well	600 µL
Elution Buffer A	Elution	5	KF plate	100 µL
Tip Comb	Tip Comb	6	Place a 96 Deep-Well Tip Comb in a Deep-Well plate	

**Refer to the table (1a or 1b) based on the heating block and plates being used. 96 Deep-Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution requires a heating step. Using incompatible plates can lead to reduced RNA yield.*

Norgen's Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **DNA Removal Protocol** is provided for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710 or 25720) be used for this step. Prepare a DNase working solution as mentioned below when using DNase treatment with the extraction.

i. For every DNase reaction to be performed, prepare a **DNase I Mix** of 15 µL of **DNase I** and 100 µL of **Enzyme Incubation Buffer A** using Norgen's RNase-Free DNase I Kit. Mix gently by inverting the tube a few times. **DO NOT VORTEX.**

Note: If using an alternative DNase I, prepare a working stock of 0.25 Kunitz/µL RNase-free DNase I solution according to the manufacturer's instructions. A 100 µL aliquot is required for each sample to be treated. Set-up the plates as per **Table 2a or 2b** when performing DNase treatment of the samples.

Table 2a. Setup of plates while using KingFisher™ Flex with a 96 Deep-Well Head and a Deep-Well heating block – *with DNase treatment.*

Set-up while using 96 Deep-Well heating block with 96 Deep-Well Head*				
Component	**Plate ID**	**Location on Carousel**	**Plate Type**	**Amount**
Lysate	Sample	1	deep-well	Variable
DNase I Mix	DNase I	2	deep-well	100 µL
Wash Solution A	Wash 1	3	deep-well	600 µL
Wash Solution A	Wash 2	4	deep-well	600 µL
Wash Solution A	Wash 3	5	deep-well	600 µL
Elution Buffer A	Elution	6	deep-well	100 µL
Tip Comb	Tip Comb	7	Place a 96 Deep-Well Tip Comb in a Deep-Well plate	

**Refer to the table (2a or 2b) based on the heating block and plates being used. 96 Deep-Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution requires a heating step. Using incompatible plates can lead to reduced RNA yield.*

Table 2b. Setup of plates while using KingFisher™ Flex with a 96 Deep-Well Head and a 96 KF heating block - *with DNase treatment.*

Set-up while using 96 KF heating block with 96 Deep-Well Head*				
Component	**Plate ID**	**Location on Carousel**	**Plate Type**	**Amount**
Lysate	Sample	1	deep-well	Variable
DNase I Mix	DNase I	2	deep-well	100 µL
Wash Solution A	Wash 1	3	deep-well	500 µL
Wash Solution A	Wash 2	4	deep-well	500 µL
Wash Solution A	Wash 3	5	deep-well	500 µL
Elution Buffer A	Elution	6	KF plate	100 µL
Tip Comb	Tip Comb	7	Place a 96 Deep-Well Tip Comb in a Deep-Well plate	

**Refer to the table (2a or 2b) based on the heating block and plates being used. 96 Deep-Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution requires a heating step. Using incompatible plates can lead to reduced RNA yield.*

Section 1. Lysate Preparation from Various Sample Types

Notes Prior to Use

- Fresh or frozen tissues may be used for the procedure.
- Tissues should be immediately frozen and stored at -20°C or -70°C. Tissues may be stored at -70°C for several months.
- It is recommended that no more than 20 mg of tissue be used. For specific tissue type, refer to **Table 3**.

1A. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use

- Cells grown in suspension or monolayer may be used.
- The maximum recommended input of cells is 3 x 10^6. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10^6 cells.
- Cell pellets can be stored at -70°C for later use or used directly in the procedure. Determine the number of cells present before freezing.

- Frozen cell pellets should not be subjected to repeated cycles of freeze and thaw prior to beginning the protocol.
- Frozen cell pellets should not be thawed prior to beginning the protocol. Add the Buffer RL directly to the frozen cell pellet **(Step 1A (ii) c).**

1A (i). Cell Lysate Preparation from Cells Growing in a Monolayer

a) Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
b) Add 350 µL of **Buffer RL** directly to culture plate.
c) Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
d) Transfer lysate to **Sample Plate**.
e) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

Note: For input amounts greater than 10^6 cells, it is recommended that the lysate is passed through a 25-gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading into the well.

1A (ii). Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

a) Transfer cell suspension to an RNase-free tube and centrifuge at no more than 200 x g (~2,000 RPM) for 10 minutes to pellet cells.
b) Carefully decant the supernatant. A few µL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
c) Add 350 µL of **Buffer RL** to the pellet and dissolve the pellet by mixing with a pipette. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
d) Transfer lysate to **Sample Plate**.
e) Proceed to **Section 2:** RNA Isolation from all Types of Lysates.

Note: For input amounts greater than 10^6 cells, it is recommended that the lysate is passed through a 25-gauge needle attached to a syringe 5-10 times at this point, in order to shear the sample contents.

If the volume of cell suspension is 1 mL or less, **steps a – c** can be performed in **Sample Plate.** Make sure to seal the plate with an Adhesive Film. In this case, proceed to **Section 2** directly after performing **step c**.

1B. Lysate Preparation from Animal Tissues

Notes Prior to Use

- Norgen's Total RNA Purification Kit (Magnetic Bead System) is designed for isolating RNA from small amount of tissue sample (up to 10 mg in most cases). If a larger amount of starting material is desired, Norgen's Animal Tissue RNA Purification Kit (Cat. #25700) should be used.
- RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Thus, it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation step.
- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. When isolating total RNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to grinding with the mortar and pestle.
- Tissues stored in RNA stabilization reagents such as RNA Preserve (Norgen Cat. 17265) or RNAlater® are compatible with this isolation procedure. Prior to isolation, carefully remove the tissue from the storage reagent using forceps, and dry excessive liquid.
- The maximum recommended input of tissue varies depending on the type of tissue being used. Please refer to Table 3 below as a guideline for maximum tissue input amounts. If your tissue of interest is not included in the table below, we recommend starting with an input of no more than 10 mg.

Table 3. Recommended Maximum Input Amounts of Different Tissues

Tissue	Maximum Input Amount
Brain	25 mg
Heart	5 mg
Kidney	10 mg
Liver	10 mg
Lung	10 mg
Spleen	10 mg

1B. Cell Lysate Preparation from Animal Tissues

a) Excise the tissue sample from the animal.
b) Determine the amount of tissue by weighing. Please refer to **Table 3** for the recommended maximum input amounts of different tissues. For tissues not included in the table, we recommend starting with an input of no more than 10 mg.
c) Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.
d) Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
e) Add 600 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate 5 – 10 times through a 25-gauge needle attached to a syringe.
f) Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
g) Spin the lysate for 2 minutes to pellet any cell debris and transfer 500 µL lysate to **Sample Plate.**
h) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

1C. Lysate Preparation from Blood

Notes Prior to Use

- This procedure is for the isolation of RNA from whole blood. ***For the isolation of RNA from plasma or serum samples, please see Section 1J.***
- Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.
- It is recommended that no more than 100 µL of blood in order not to saturate the magnetic beads.
- We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA as the anti-coagulant.
- It is important to work quickly during this procedure.

1C. Lysate Preparation from Blood

a) Transfer up to 100 µL of non-coagulated blood to **Sample Plate.**
b) Add 350 µL of **Buffer RL** to the blood.
c) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

1D. Lysate Preparation from Nasal or Throat Swabs

Notes Prior to Use

- Body fluid of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with these samples.
- It is important to work quickly during this procedure.

1D. Lysate Preparation from Nasal or Throat Swabs

a) Add 600 µL of **Buffer RL** to an RNase-free microcentrifuge tube (not provided).

b) Gently brush a sterile, single-use cotton swab inside the nose or mouth of the subject.
c) Using sterile techniques cut the cotton tip where the nasal or throat cells were collected and place into the microcentrifuge tube containing the Buffer RL. Close the tube. Vortex gently and incubate for 5 minutes at room temperature.
i) Transfer 500 µL lysate to **Sample Plate**.

Note: In case of swab samples collected in Norgen's Total Nucleic Acid Preservation Tubes (Cat# 69200) transfer 500 µL preserved sample directly to **Sample Plate** and proceed to **Section 2**.

j) Proceed to **Section 2**: RNA Isolation from All Types of Lysates.

1E. Lysate Preparation from Bacteria

Notes Prior to Use

- Prepare the appropriate lysozyme-containing TE Buffer as indicated in **Table 4.** This solution should be prepared with sterile, RNase-free TE Buffer, and kept on ice until needed. These reagents are to be provided by the user.
- It is recommended that no more than 10^9 bacterial cells be used in this procedure. Bacterial growth can be measured using a spectrophotometer. As a general rule, an *E. coli* culture containing 1 x 10^9 cells/mL has an OD_{600} of 1.0.
- For RNA isolation, bacteria should be harvested in log-phase growth.
- Bacterial pellets can be stored at -70°C for later use, or used directly in this procedure.
- Frozen bacterial pellets should not be thawed prior to beginning the protocol. Add the Lysozyme-containing TE Buffer directly to the frozen bacterial pellet **(Step 1Ec).**

1E. Lysate Preparation from Bacteria

a) Pellet bacteria by centrifuging at 14,000 x g (~14,000 RPM) for 1 minute. (***Note:*** Centrifuge at 4000 rpm for 3 minutes while centrifuging bacterial suspension in a 96-well plate).
b) Decant supernatant, and carefully remove any remaining media by aspiration.
c) Resuspend the bacteria thoroughly in 100 µL of the appropriate lysozyme-containing TE buffer by vortexing. Incubate at room temperature for the time indicated in **Table 4**.
d) Add 350 µL of **Buffer RL** to the pellet and dissolve the pellet by mixing with a pipette. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
e) Transfer lysate to **Sample Plate.**

Note: If the volume of bacterial suspension is 1 mL or less, **steps a – d** can be performed in **Sample Plate.** Make sure to seal the plate with an Adhesive Film. In this case proceed to **Section 2** directly after performing **step d**.

f) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

Table 4. Incubation Time for Different Bacterial Strains

Bacteria Type	Lysozyme Concentration in TE Buffer	Incubation Time
Gram Negative	1 mg/mL	5 min
Gram Positive	3 mg/mL	10 min

1F. Lysate Preparation from Yeast

Notes Prior to Use

- Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that 100 µL of buffer is required for each preparation. The Resuspension Buffer should have the following composition: 50 mM Tris, pH 7.5, 10 mM EDTA, 1M Sorbitol, 0.1% β-mercaptoethanol and 1 unit/ µL Lyticase. This solution should be prepared with sterile, RNAse-free reagents, and kept on ice until needed. These reagents are to be provided by the user.
- It is recommended that no more than 10^7 yeast cells or 1 mL of culture be used for this procedure.

- For RNA isolation, yeast should be harvested in log-phase growth.
- Yeast can be stored at -70°C for later use, or used directly in this procedure.
- Frozen yeast pellets should not be thawed prior to beginning the protocol. Add the Lyticase-containing Resuspension Buffer directly to the frozen yeast pellet **(Step 1Fc).**

1F. Lysate Preparation from Yeast

g) Pellet yeast by centrifuging at 14,000 x g (~14,000 RPM) for 1 minute. (***Note:*** Centrifuge at 4000 rpm for 3 minutes while centrifuging bacterial suspension in a 96-well plate).
a) Decant supernatant, and carefully remove any remaining media by aspiration.
b) Resuspend the yeast thoroughly in 100 µL of Lyticase-containing Resuspension Buffer by vortexing.
c) Incubate at 37ºC for 10 minutes.
d) Add 350 µL of **Buffer RL** to the pellet and dissolve the pellet by mixing with a pipette. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
e) Transfer lysate to **Sample Plate.**

Note: If the volume of yeast suspension is 1 mL or less, **step a – e** can be performed in **Sample Plate.** Make sure to seal the plate with an Adhesive Film. In this case proceed to **Section 2** directly after performing **step e**.

f) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

1G. Lysate Preparation from Fungi

Notes Prior to Use

- Fresh or frozen fungi may be used for this procedure. Fungal tissues should be flash frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Fungi may be stored at -70°C for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
- It is recommended that no more than 50 mg of fungi be used for this procedure in order not to saturate the magnetic beads.

1G. Lysate Preparation from Fungi

a) Determine the amount of fungi by weighing. It is recommended that no more than 50 mg of fungi be used for the protocol.
b) Transfer the fungus into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the fungus thoroughly using a pestle.

Note: At this stage the ground fungus may be stored at -70°C, such that the RNA purification can be performed at a later time.

c) Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
d) Add 600 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.
e) Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
f) Spin the lysate for 2 minutes to pellet any cell debris. Transfer 500 µL lysate to **Sample Plate.**
g) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

1H. Lysate Preparation from Plant

Notes Prior to Use

- The maximum recommended input of plant tissue is 50 mg or 5 x 10^6 plant cells.
- Both fresh and frozen plant samples can be used for this protocol. Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
- It is important to work quickly during this procedure.

1H. Lysate Preparation from Plant

a) Determine the amount of plant by weighing and transfer ≤50 mg plant tissue or 5 x 10^6 plant cells into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the sample into a fine powder using a pestle in liquid nitrogen.

Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen.

b) Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
c) Add 600 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.
d) Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided) Spin the lysate for 2 minutes to pellet any cell debris. Transfer up to 500 µL supernatant to **Sample Plate.**
e) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

1I. Lysate Preparation from Viral Suspension

Notes Prior to Use

- For the isolation of integrated viral RNA, follow **Section 1A** if the starting material is tissue, follow **Section 1B** if the starting material is cell culture, follow **Section 1C** if the starting material is blood, or follow **Section 1D** if the starting material is a nasal or throat swab.
- For the isolation of RNA from free viral particles, follow the procedure below.
- It is recommended that no more than 100 µL of viral suspension be used in order to perform effective isolation.
- It is important to work quickly during this procedure.

1I. Lysate Preparation from Viral Suspension

a) Transfer 100 µL of viral suspension to **Sample Plate**.
b) Add 350 µL of **Buffer RL**.
c) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

1J. Lysate Preparation from Plasma or Serum

Notes Prior to Use

- Plasma or Serum of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with plasma or serum.
- We recommend the use of this kit to isolate RNA from plasma or serum prepared by standard protocol from non-coagulating fresh blood using EDTA or sodium citrate as the anti-coagulant.
- Plasma prepared from fresh blood using heparin as an anti-coagulant is not suitable for use with this protocol. For heparin-prepared samples follow the protocol in section 1C, Lysate Preparation from Blood.
- It is recommended that no more than 200 µL of plasma or serum be used in order to prevent saturation of magnetic bead.
- Avoid multiple freeze-thaw cycle of the plasma or serum sample. Aliquot to the appropriate volume for usage prior to freezing.
- It is important to work quickly during this procedure.

1J. Lysate Preparation from Plasma or Serum

a) Transfer 100 µL of plasma or serum to **Sample Plate**.
b) Add 350 µL of **Buffer RL.**
c) **Optional:** Add 0.7 µL of 0.8 µg/µl MS2 RNA per sample.

Note: The use of MS2 RNA could increase the consistency of downstream applications such as RT-PCR. However, the use of MS2 RNA is not recommended for applications involving global gene expression analysis such as microarrays or sequencing.

d) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

1K. Lysate Preparation from Fresh Saliva Samples

Notes Prior to Use

- Bodily fluids of all human and animal subjects are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with these samples.
- It is important to work quickly during this procedure.

1K. Lysate Preparation from Fresh Saliva.

a) Transfer 100 µL of fresh saliva to **Sample Plate**.
b) Vortex the preserved saliva sample for 10 seconds.
c) Add 350 µL of **Buffer RL.**
d) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

1L. Lysate Preparation from Norgen's Saliva RNA Collection and Preservation Devices (RU53800, and 53800, RU69800, and 69800).

Notes Prior to Use

- Bodily fluids of all human and animal subjects are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with these samples.
- It is important to work quickly during this procedure.

1L. Lysate Preparation from Norgen's Saliva RNA Collection and Preservation Devices (RU53800, and 53800, RU69800, and 69800).

a) Collect saliva into Norgen's Saliva RNA Collection and Preservation Device according to the manufacturer's instructions.
b) Refer to manufacturers' instructions for storage and shipment conditions.
c) Vortex the preserved saliva sample for 10 seconds.
d) Transfer 200 µL of preserved saliva to **Sample Plate**.
e) Add 250 µL of **Buffer RL.**
f) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

Section 2: Total RNA Purification from All Types of Lysates

Table 5. Isopropanol and Magnetic Beads C Volume for Each Sample Type

Section	Sample Type	Lysate Volume (µL)	Isopropanol (µL)	Magnetic Beads C (µL)
1A (i)	Cells (Monolayer)	350	350	40
1A (ii)	Cells (Suspension)	350	350	40
1B	Animal Tissues	500	500	40
1C	Blood	450	450	40
1D	Nasal or Throat Swabs	500	500	40
1E	Bacteria	350	350	40
1F	Yeast	350	350	40
1G	Fungi	500	500	40
1H	Plant	500	500	40
1I	Viral Suspension	450	450	40
1J	Plasma or Serum	450	450	40
1K	Fresh Samples	450	450	40
1L	Preserved Saliva	450	450	40

Table 6. KingFisher™ Flex scripts to be used while working with KingFisher™ 96 Deep-Well Head (Cat. 5400630).

Heating Block	Elution Plate	DNase I Treatment	KingFisher Script
96 Deep-Well (24075430)	Deep-Well 96 plate (95040450 or 95040460)	No	TOTRNA96_KF_DW
		Yes	TOTRNA96DNA_KF_DW
96 KF (24075420)	96 KF plate (97002540)	No	TOTRNA96_KF
		Yes	TOTRNA96DNA_KF

2.1 Total RNA Purification using KingFisher™ Flex system

a) Setup plates according to **Table 1a** or **1b.** Place a clean tip comb in an empty plate **(Tip Comb plate).**
b) Add 100 µl of **Elution Buffer A** to the **Elution plate**.
c) Add 600 µL of **Wash Solution A** to **Wash Plate 1, Wash plate 2, and Wash plate 3.**
d) After setting up, run the program **TOTRNA96_KF_DW** or **TOTRNA96_KF** based on **Table 6**.
e) Place the plates as promoted by the KingFisher™ Flex instrument.
f) Prepare a mixture of **Isopropanol** and 40 µL **Magnetic Beads C** as per **Table 5** for the number of samples extracted. (For example, if RNA is to be extracted from 10 samples, and if the lysate volume obtained in **Section 1** is 500 µL, mix 5 mL of **Isopropanol** with 400 µL **Magnetic Beads C** and add 540 µL of the mixture to each well of **Plate 2**). A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.
g) After few minutes of incubation, the instrument will pause and will allow the user to add mixture of ethanol and magnetic beads prepared in **step f.** Open the instrument carousel door and remove the plate from the instrument and add the prepared mixture from **step f** to **Sample Plate**.
h) Put the plate back into the instrument and follow the instrument prompt. The instrument will continue to perform the extraction.
i) The instrument will prompt after the procedure is complete. Remove the **Elution plate** from the instrument and the RNA is now ready for further downstream processing.
j) The purified RNA sample may be stored at -20°C for a few days. The provided Adhesive Tape can be used to seal the **Elution plate** for storage of the RNA. It is recommended that samples be placed at -70°C for long-term storage.

2.2 Total RNA Purification using IsoPureTM 96 system – with DNase Treatment

a) Setup plates according to **Table 2a** or **2b.** Place a clean tip comb in an empty plate **(Tip Comb plate).**
b) Add 100 µl of **Elution Buffer A** to the **Elution plate**.
c) Add 600 µL of **Wash Solution A** to **Wash plate 1, Wash plate 2, and Wash plate 3.**
d) Add 100 µL of **DNase I Mix** to **DNase I plate.**
e) After setting up, run the program **TOTRNA96DNA_KF_DW** or **TOTRNA96DNA_KF** based on **Table 6**.
f) Place the plates as promoted by the KingFisher™ Flex instrument.
g) Prepare a mixture of **Isopropanol** (not provided) and 40 µL of **Magnetic Beads C** as per **Table 5** for the number of samples extracted. (For example, if RNA is to be extracted from 10 samples, and if the lysate volume obtained in **Section 1** is 500 µL, mix 5 mL of **Isopropanol** (not provided) with 400 µL **Magnetic Beads C** and add 540 µL of the mixture to each well of **Sample plate**). A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.
h) After few minutes of incubation, the instrument will pause and will allow the user to add mixture of ethanol and magnetic beads prepared in **step g.** Open the instrument carousel door and remove the plate from the instrument and add the prepared mixture from **step g** to **Sample Plate**.
i) Put the plate back into the instrument and follow the instrument prompt. The instrument will continue to perform the extraction.
j) To rebind the sample to the magnetic beads, prepare a mixture of 200 µL **Buffer RL** and 300 µL Isopropanol (not provided) for the number of samples extracted. (For example, if RNA is to be extracted from 10 samples, and mix 2 mL of **Buffer RL** with 3 mL **Isopropanol**). A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.

k) After a few minutes, the instrument will pause and allow the user to add mixture prepared in **step j**. Open the instrument carousel door and remove the plate from the instrument and add the prepared mixture from **step g** to **DNase I Plate**.
l) Put the plate back into the instrument and follow the instrument prompt. The instrument will continue to perform the extraction.
m) The instrument will prompt after the procedure is complete. Remove the **Elution plate** from the instrument and the RNA is now ready for further downstream processing.
n) The purified RNA sample may be stored at -20°C for a few days. The provided Adhesive Tape can be used to seal the **Elution plate** for storage of the RNA. It is recommended that samples be placed at -70°C for long-term storage.

Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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