PRODUCT INFORMATION

Thermo Scientific MagJET RNA Kit

#K2731, #K2732

Read Storage information (p. 4) upon receipt and store kit components appropriately!

www.thermoscientific.com/onebio

#K2731, #K2732
Lot 00000000
Expiry Date 00.0000

CERTIFICATE OF ANALYSIS

Thermo Scientific™ MagJET™ RNA Kit is qualified by isolating RNA from 5 mg of frozen mouse muscle or heart following the protocol outlined in the manual. The quality of isolated RNA is evaluated spectrophotometrically and by agarose gel electrophoresis. The purified RNA has an A_{260}/A_{280} ratio of 2.0±0.3. The functional quality of purified RNA is evaluated by RT-PCR.

Quality authorized by:  **Jurgita Žilinskienė**

Rev.3 45

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COMPONENTS OF THE KIT

MagJET RNA Kit	#K2731 96 preps	#K2732 384 preps
Lysis Buffer for MagJET RNA Kit	50 mL	200 mL
MagJET Magnetic Beads	3 × 1.4 mL	2 × 8.5 mL
DNase I (lyophilized)	1 vial	1 vial
10X Reaction Buffer with $MgCl_2$ for DNase I	3 × 1 mL	9 × 1 mL
DNase I Reconstitution Buffer	1 mL	2.5 mL
Wash Buffer 1 (conc.) for MagJET RNA Kit	40 mL	2 × 80 mL
Wash Buffer 2 (conc.) for MagJET RNA Kit	45 mL	3 × 45 mL
Water, nuclease-free	30 mL	125 mL

STORAGE

DNase I (lyophilized), 10X Reaction Buffer with $MgCl_2$ for DNase I and DNase I Reconstitution Buffer should be stored at -20°C upon arrival. Reconstituted DNase I should be stored at -20°C. MagJET Magnetic Beads should be stored at 4°C. Other components of the kit should be stored at room temperature (15-25°C).

DESCRIPTION

The MagJET RNA Kit is designed for fast and efficient purification of total RNA from cultured mammalian cells, tissues, bacteria and yeast.
The kit utilizes paramagnetic bead technology enabling high yields and robust performance. High binding capacity, uniform particle size, and rapid magnetic response of MagJET magnetic beads makes the technology ideal for high throughput automatic nucleic acid purification, as well as for manual nucleic acid purification by low sample throughput users.
The resulting high quality purified RNA is free of proteins, nucleases and other contaminants or inhibitors, can be used in a wide range of downstream applications, such as RT-PCR, RT-qPCR and other enzymatic reactions. *See* Table 1 for typical total RNA yields from various sources.

PRINCIPLE

The MagJET RNA Kit uses the highly efficient MagJET magnetic particle based-technology for nucleic acid purification. The whole nucleic acid isolation process combines simple steps of sample lysis, RNA binding to the magnetic beads, DNA removal, washing and elution. Purification protocols optimized for automated KingFisher instruments utilize high throughput magnetic bead transfer technique, where magnetic beads are transferred through different reagent plates containing lysis, binding, washing and elution reagents. This enables high throughput nucleic acid purification and eliminates multiple pipetting steps.
Alternatively, protocol is provided where instead of magnetic particles, buffers and other reagents are transferred in each of the protocol steps, while magnetic beads remain captured on the wall of the tube using a magnetic rack. This allows the kit to be used in various throughput applications using a magnetic rack and manual or automated pipetting equipment.

Table 1. Typical total RNA yields from various sources.

Source	Quantity	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

IMPORTANT NOTES

- Add the indicated volume of ethanol (96-100%) to **Wash Buffer 1** (conc.) and **Wash Buffer 2** (conc.) prior to first use:

	96 preps		384 preps	
	Wash Buffer 1	**Wash Buffer 2**	**Wash Buffer 1**	**Wash Buffer 2**
Concentrated buffer	40 mL	45 mL	80 mL	45 mL
Ethanol (96-100%)	**40 m**L	**180 m**L	**80 mL**	**180 mL**
Total volume:	80 mL	225 mL	160 mL	225 mL

After preparing each solution, mark the bottle to indicate that this step has been completed.

- To prepare the **DNase I** solution add 0.55 mL (Cat # K2731 - 96 preps kit) or 2.2 mL (Cat # K2732 - 384 preps kit) of **DNase I Reconstitution Buffer** to each vial and incubate at room temperature for 5 minutes. Occasional gentle rotation of the vial helps to dissolve the DNase I, but avoid forceful mixing. Do not vortex! Store at -20°C.
- To prepare **1X Reaction Buffer with $MgCl_2$ for DNase I** for purification of 1 sample, mix 20 µL 10X Reaction Buffer with $MgCl_2$ for DNase I and 180 µL of nuclease-free water. It is recommended to use freshly prepared buffer.
- Check all solutions for any salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37°C, and then equilibrate to room temperature (15-25°C).
- Wear gloves when handling the **Lysis Buffe**r **and Wash Buffer 1** as these reagents contain irritants (*see* p.20 for SAFETY INFORMATION).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and RNase-free pipette tips.
- Thermomixer.
- Vortex.
- Microcentrifuge.
- 1.5 mL tubes, RNase-free.
- Disposable gloves.
- 96-100% ethanol, molecular biology grade.
- 2 M DTT or 14.3 M β-mercaptoethanol.
- Equipment for sample disruption and homogenization (depending on the method chosen):
  - Mortar and pestle.
  - Homogenizer.
  - Automatic magnetic particle processor and consumables *or*
  - Magnetic rack.

Buffers

- For mammalian cultured cells lysate preparation:
  PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4).
- For bacterial lysate preparation:
  TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA) containing lysozyme (0.4 mg/mL final concentration).
- For yeast lysate preparation:
  Yeast lysis buffer (1 M sorbitol, 0.1 M EDTA, pH 7.4. Add 0.1% -β-mercaptoethanol and 5 mg/mL of Lyticase or Zymolyase 20T just prior to use).

AVOIDING RIBONUCLEASE CONTAMINATION

RNA purity and integrity is essential for downstream applications. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. Care must be taken not to introduce RNases into the RNA preparation, especially during the wash with Wash Buffer 2 and elution steps.

General recommendations to avoid RNase contamination:

- Wear gloves when handling reagents and RNA samples, as skin is a common source of RNases. Change gloves frequently.
- Use sterile, disposable RNase-free pipette tips.
- Use appropriate reagents to remove RNase contamination from non-disposable items such as pipettes, centrifuges and work surfaces.
- Keep all kit components tightly sealed when not in use. After use close bottles immediately.

STARTING MATERIAL HANDLING AND STORAGE

- When purifying RNA from fresh samples, place the samples in liquid nitrogen immediately after harvesting. Proceed to lysis and homogenization as quickly as possible.
- When samples are obtained from sacrificed animals or cadavers, limit the time between death and sample collection to isolate high quality RNA.
- If RNA is not to be purified immediately after tissue collection, freeze the samples in liquid nitrogen and store at -70°C. Frozen tissue samples should not be allowed to thaw during handling or weighing.
- Mammalian cultured cells can be pelleted and stored at -70°C until required.
- For RNA purification from yeast cells using enzymatic lysis, only freshly harvested samples can be used.

PROTOCOL SELECTION GUIDE

The MagJET RNA Kit provides optimized protocols for total RNA purification from mammalian cultured cells, tissue, bacteria and yeast. The kit is compatible with automated and manual processing.

The following selection guide summarizes available protocols depending on the source of starting material, throughput and sample processing type. Automation protocols are optimized for KingFisher Flex and KingFisher Duo instruments.

Note: Transfer the **Tissue_RNA_Flex** protocol file to the KingFisher Flex or **Tissue_RNA_Duo** protocol file to the KingFisher Duo instrument before first use. 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. The protocol files for MagJET RNA Kit can be found on product web page on www.thermoscientific.com/onebio.

Protocol selection guide:

Sample type	Sample quantity	Throughput per run	KingFisher Flex Instrument	KingFisher Duo Instrument	Manual processing	MagJET purification protocol	Page
Mammalian cell culture	up to 2×10^6 cells	96	●	-	-	**Protocol A**	page 8
		12	-	●	-	**Protocol C**	page 12
		variable	-	-	●	**Protocol E**	page 15
Tissue	up to 20 mg	96	●	-	-	**Protocol B**	page 11
		12	-	●	-	**Protocol D**	Page 14
		variable	-	-	●	**Protocol E**	Page 15
Bacterial culture	up to 10^9 cells	96	●	-	-	**Protocol F**	page 17
		12	-	●	-		
		variable	-	-	●		
Yeast culture	up to 10^8 cells	96	●	-	-	**Protocol G**	page 18
		12	-	●	-		
		variable	-	-	●		

TOTAL RNA PURIFICATION PROTOCOLS AND PIPETTING INSTRUCTIONS

Protocol A. Instructions for total RNA purification from up to 2 × 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates

Before starting:

- Transfer the Tissue_RNA_Flex protocol file to the KingFisher Flex as described on page 7.
- Supplement the required amount of Lysis Buffer with DTT or β-mercaptoethanol. Add 10 µL 2 M DTT or 10 µL 14.3 M β-mercaptoethanol to each 450 µL volume of Lysis Buffer required.

Note. When using the MagJET RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Reconstitute DNase I (lyophilized) in DNase I Reconstitution Buffer as described on page 5.
- Prepare 1X Reaction Buffer with $MgCl_2$ for DNase I as described in the page 5.

1. Lysis of Cultured Mammalian Cells:

   a) Suspension cells
   Pellet up to 2×10^6 cells in an appropriate centrifuge tube for 5 min at 300 × g. Discard the supernatant. Rinse the cells once with PBS to remove residual growth medium. Repeat centrifugation step and discard the supernatant.

   b) Adherent cells
   Remove the growth medium from the cells (use up to 2×10^6 cells). Rinse the cells once with PBS to remove residual medium. Remove and discard PBS. Detach the cells from the culture plate by scraping in appropriate volume of PBS or by trypsinization. Transfer the cells into microcentrifuge tube (not included) and pellet them by centrifugation for 5 min at 300 × g. Discard the supernatant.

2. Obtain five empty Thermo Scientific Microtiter deep well 96 plates and two empty Thermo Scientific KingFisher 96 KF plates.

3. Resuspend collected cells in 450 µL of **Lysis Buffer** supplemented with **DTT or β-mercaptoethanol**. Mix by vortexing to obtain a uniform suspension. Spin down the tubes to collect all the drops from the walls of the tube. Transfer the prepared lysate to the **Sample** plate (Microtiter deep well 96 plate).

4. Prepare the plates as follows:

Plate number	Plate type	Plate name	Content	Volume per well
2*	Microtiter deep well 96 plate	DNase I	1X Reaction Buffer with $MgCl_2$ for DNase I	200 µL
			DNase I (reconstituted)	5 µL
3		Wash 1	Wash Buffer 1 (*supplemented with ethanol*)	700 µL
4		Wash 2_1	Wash Buffer 2 (*supplemented with ethanol*)	700 µL
5		Wash 2_2	Wash Buffer 2 (*supplemented with ethanol*)	700 µL
6	KingFisher Flex 96 KF plate	Elution	Water, nuclease free	100 µL
7		Tip Plate	-	-

* For better results, avoid storage of DNase I in 1X Reaction Buffer with $MgCl_2$ for DNase I at roomtemperature for extended periods of time. It is recommended to prepare the **DNase I** plate the last.

5. Add the following reagents to the **Sample** plate.

Plate number	Plate type	Plate name	Content	Volume per well
1	Microtiter deep well 96 plate	Sample	Lysed sample	450 µL
			Magnetic Beads*	40 µL
			Ethanol	400 µL

* Resuspend Magnetic Beads well by vortexing before use.

6. Place a Thermo Scientific KingFisher Flex 96 tip comb for deep well magnets on a **Tip Plate** (KingFisher Flex 96 KF plate).
7. Start the **Tissues_RNA_Flex** protocol on the KingFisher Flex 96 and load the plates according to the KingFisher display. After all the plates have been loaded into the instrument, the protocol will begin.

8. When the KingFisher Flex pauses at the dispense step after the DNase I digestion step (approximately 25 minutes after starting the run), remove the **DNase I** plate from the instrument and add 200 µL of the ethanol (96-100%) per well to the **DNase** I plate to rebind the RNA.

Plate number	Plate type	Plate name	Content	Volume per well
2	Microtiter deep well 96 plate	DNase I	Ethanol	200 µL

9. Place the **DNase I** plate back into the instrument and press **Start**. After the pause, the protocol will continue to the end.
10. When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and turn off the instrument. Transfer the eluate (which contains the purified RNA) to a new, sterile tube and close immediately. The purified RNA is ready for use in downstream applications. Keep the purified RNA on ice for immediate use, or store at -20°C or -70°C.

Protocol B. Instructions for total RNA purification from up to 20 mg of tissue using KingFisher Flex 96 and Microtiter deep well 96 plates

Before starting:

- Transfer the Tissue_RNA_Flex protocol file to the KingFisher Flex as described on page 7.
- Supplement the required amount of Lysis Buffer with DTT or β-mercaptoethanol. Add 10 µL 2 M DTT or 10 µL 14.3 M β-mercaptoethanol to each 450 µL volume of Lysis Buffer required.

Note. When using the MagJET RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Reconstitute DNase I (lyophilized) in DNase I Reconstitution Buffer as described on page 5.
- Prepare 1X Reaction Buffer with $MgCl_2$ for DNase I as described on page 5.

1. Lysis of mammalian tissues samples:
Weigh the tissue (use up to 20 mg of fresh or frozen tissue) and disrupt the material by one of the following methods:

   a) Disruption using a mortar and pestle:
   Place up to 20 mg of tissue (use up to 10 mg of spleen tissue) into liquid nitrogen and grind thoroughly with mortar and pestle. Transfer the tissue powder immediately into a 1.5 mL microcentrifuge tube (not included) containing 450 µL of **Lysis Buffer** supplemented with **DTT** or **β-mercaptoethanol**. Mix by vortexing to obtain a uniform suspension. Spin down the tube to collect all the drops from the walls of the tube.

   Note:

   - Transfer the tissue powder to the Lysis Buffer as quickly as possible. Leaving the powder without the Lysis Buffer can result in degraded RNA.
   - The homogenized tissue should be directly used for RNA purification and should not be stored.
   - All homogenized material must be thoroughly mixed with the Lysis Buffer and should not be allowed to dry on the walls of the tube (this can cause degradation of RNA).

   b) Disruption and homogenization using homogenizer:
   Homogenize the tissue according manufacturer recommendations.

2. Obtain five empty Thermo Scientific Microtiter deep well 96 plates and two empty Thermo Scientific KingFisher Flex 96 KF plates.
3. Transfer the prepared lysate to the **Sample** plate (Microtiter deep well 96 plate).
4. Proceed to **Step 4** of the **Protocol A: Instructions for total RNA purification from up to 2×10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates**" on page 9 for the further purification.

Protocol C. Instructions for total RNA purification from up to 2 × 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates

Before starting:

1. Supplement the required amount of Lysis Buffer with DTT or β-mercaptoethanol. Add 10 µL 2 M DTT or 10 µL 14.3 M β-mercaptoethanol to each 450 µL volume of Lysis Buffer required.
2. Transfer the **Tissue_RNA_Duo** protocol file to the KingFisher Duo as described on page 7. Ensure you are using the KingFisher Duo 12-pin magnet head and heating block.

Note. When using the MagJET RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Reconstitute DNase I (lyophilized) in DNase I Reconstitution Buffer as described on page 5.
- Prepare 1X Reaction Buffer with $MgCl_2$ for DNase I as described on page 5.

1. Lysis of Cultured Mammalian Cells:

   a) Suspension cells
   Pellet up to 2×10^6 cells in an appropriate centrifuge tube for 5 min at 300 × g. Discard the supernatant. Rinse the cells once with PBS to remove residual growth medium. Repeat centrifugation step and discard the supernatant.

   b) Adherent cells
   Remove the growth medium from the cells (use up to 2×10^6 cells). Rinse the cells once with PBS to remove residual medium. Remove and discard PBS. Detach the cells from the culture plate by scraping in appropriate volume of PBS or by trypsinization. Transfer the cells into microcentrifuge tube (not included) and pellet them by centrifugation for 5 min at 300 × g. Discard the supernatant.

2. Resuspend collected cells in 450 µL **Lysis Buffer** supplemented with **DTT** or **β-mercaptoethanol**. Mix by vortexing to obtain a uniform suspension. Spin down the tubes to collect all the drops from the walls of the tube.
3. Obtain one empty Thermo Scientific Microtiter deep well 96 plate and one Thermo Scientific KingFisher Duo elution strip. Transfer the prepared lysate to **row B** of the **RNA** plate.

4. Prepare the **RNA plate** (Microtiter deep well 96 plate) according to the instructions below. Add the following reagents to the rows. **Note that row G is reserved for the tip and should be left empty. Note that rows F and H are left empty.**

Plate name and type	Row	Row name	Content	Volume per well
RNA plate Microtiter deep well 96 plate	A*	DNase I*	1X Reaction Buffer with $MgCl_2$ for DNase I	200 µL
			DNase I (reconstituted)	5 µL
	B	Sample**	Lysed Sample	450 µL
			Magnetic Beads (*resuspended before use*)	40 µL
			Ethanol	400 µL
	C	Wash 1	Wash buffer 1 (*supplemented with ethanol*)	700 µL
	D	Wash 2_1	Wash buffer 2 (*supplemented with ethanol*)	700 µL
	E	Wash 2_2	Wash buffer 2 (*supplemented with ethanol*)	700 µL
	F	Empty	Empty	Empty
	G	Tip Comb	12-tip comb	---
	H	Empty	Empty	Empty

* For better results, avoid storage of DNase I in 1X Reaction Buffer with $MgCl_2$ for DNase I at room temperature for extended periods of time. It is recommended to fill **row A** the last.
** It is recommended to prepare the Sample row B after rows C-E are filled.

5. Fill the KingFisher Duo **Elution Strip** as follows:

Elution strip*	Content	Reagent volume per well
KingFisher Duo elution strip	Water, nuclease free	100* µL

* If more concentrated RNA is required, the volume of the nuclease- free water can be reduced to 50 µL. If less concentrated RNA is required the volume of the water can be increased up to 150 µL.

6. Place a Thermo Scientific KingFisher Duo 12-tip comb into **row G** of the **RNA** plate.
7. Switch on the KingFisher Duo. Start the **Tissue_RNA_Duo** protocol and load the plate and **Elution Strip** according to the KingFisher display. Ensure that the elution strip is placed in the correct direction into the elution block and that the perforated end is facing towards the user. After all plates have been loaded the program will start.
8. When the KingFisher Duo pauses at the dispense step after the DNase I digestion step (approximately 25 minutes after starting the run), remove the **RNA** plate from the instrument and add 200 µL ethanol (96-100%) per well to the **row A** to rebind the RNA.

Row	Row name	Content	Volume per well
A	DNase I	Ethanol	200 µL

9. Place the **RNA** plate back into the instrument and press **OK.** After the pause, the protocol will continue to the end.
10. After the run is completed, remove the **RNA** plate and **Elution Strip** according to the instructions on the KingFisher Duo display and turn off the instrument. Transfer the eluate (which contains the purified RNA) to a new, sterile tube and close immediately. The purified RNA is ready for use in downstream applications. Keep purified RNA on ice for immediate use, or store at -20°C or -70°C.

Protocol D. Instructions for total RNA purification from up to 20 mg tissues using KingFisher Duo and Microtiter deep well 96 plates

Before starting:

- Supplement the required amount of Lysis Buffer with DTT or β-mercaptoethanol. Add 10 µL 2 M DTT or 10 µL 14.3 M β-mercaptoethanol to each 450 µL volume of Lysis Buffer required.
- Transfer the **Tissue_RNA_Duo** protocol file to the KingFisher Duo as described on page 7. Ensure you are using the KingFisher Duo 12-pin magnet head and heating block

Note. When using the MagJET RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Reconstitute DNase I (lyophilized) in DNase I Reconstitution Buffer as described on page 5.
- Prepare 1X Reaction Buffer with $MgCl_2$ for DNase I as described on page 5.

1. Lysis of mammalian tissues samples:
   Weigh the tissue (use up to 20 mg of fresh or frozen tissue) and disrupt the material by one of the following methods:

   a) Disruption using a mortar and pestle:
   Place up to 20 mg of tissue (use up to 10 mg of spleen tissue) into liquid nitrogen and grind thoroughly with mortar and pestle. Transfer the tissue powder immediately into a 1.5 mL microcentrifuge tube (not included) containing 450 µL of **Lysis Buffer** supplemented with **DTT** or **β-mercaptoethanol**. Mix by vortexing to obtain a uniform suspension. Spin down the tubes to collect all the drops from the walls of the tube.

   Note.
   - Transfer the tissue powder to the Lysis Buffer as quickly as possible. Leaving the powder without the Lysis Buffer can result in degraded RNA.
   - The homogenized tissue should be directly used for RNA purification and should not be stored.
   - All homogenized material must be thoroughly mixed with the Lysis Buffer and should not be allowed to dry on the walls of the tube (this can cause degradation of RNA).

   b) Disruption and homogenization using homogenizer:
   Homogenize the tissue according manufacturer recommendations.

2. Obtain one empty Thermo Scientific Microtiter deep well 96 plate and one Thermo Scientific KingFisher Duo elution strip.
3. Transfer the prepared lysate into the **row B** of the **RNA** plate (Microtiter deep well 96 plate).
4. Proceed to **Step 4** of the **Protocol C: "Instructions for total RNA purification from up to 2 × 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates"** on page 13 for the further purification.

Protocol E. Instructions for manual RNA purification from up to 2 × 10^6 cultured mammalian cells and up to 20 mg tissue

Following protocol is based on transfer of liquids by pipetting through different purification steps rather than magnetic bead transfer as in KingFisher automatic protocols. This allows the kit to be used in various throughput applications using magnetic rack and manual or automated pipetting equipment. Protocols for the other automated pipetting platforms should be optimized for each platform and sample used. To enable protocol optimization all buffers are available to purchase separately.

Before starting:

- Supplement the required amount of Lysis Buffer with DTT or β-mercaptoethanol. Add 10 µL 2 M DTT or 10 µL 14.3 M β-mercaptoethanol to each 450 µL volume of Lysis Buffer required.

Note. When using the MagJET RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Reconstitute DNase I (lyophilized) in DNase I Reconstitution Buffer as described on page 5.
- Prepare 1X Reaction Buffer with $MgCl_2$ for DNase I as described on page 5.

1. Lysis of Cultured Mammalian Cells:

   a) Suspension cells
   Pellet up to 2×10^6 cells in an appropriate centrifuge tube for 5 min at 300 × g. Discard the supernatant. Rinse the cells once with PBS to remove residual growth medium. Repeat centrifugation step and discard the supernatant. Add 450 µL of **Lysis Buffer** supplemented with **DTT** or **β-mercaptoethanol**. Mix by vortexing to obtain a uniform suspension. Spin down the tubes to collect all the drops from the walls of the tube.

   b) Adherent cells
   Remove the growth medium from the cells (use up to 2×10^6 cells). Rinse the cells once with PBS to remove residual medium. Remove and discard PBS. Detach the cells from the culture plate by scraping in appropriate volume of PBS or by trypsinization. Transfer the cells into microcentrifuge tube (not included) and pellet them by centrifugation for 5 min at 300 × g. Discard the supernatant. Add 450 µL of **Lysis Buffer** supplemented with **DTT** or **β-mercaptoethanol**. Mix by vortexing to obtain a uniform suspension. Spin down the tubes to collect all the drops from the walls of the tube.

   Lysis of mammalian tissues samples:
   Weigh the tissue (use up to 20 mg of fresh or frozen tissue) and disrupt the material by one of the following methods:

   a) Disruption using a mortar and pestle:
   Place up to 20 mg of tissue (use up to 10 mg of spleen tissue) into liquid nitrogen and grind thoroughly with mortar and pestle. Transfer the tissue powder immediately into a 1.5 mL microcentrifuge tube (not included) containing 450 µL of **Lysis Buffer** supplemented with **DTT** or **β-mercaptoethanol**. Mix by vortexing to obtain a uniform suspension. Spin down the tubes to collect all the drops from the walls of the tube.

   Note.

   - Transfer the tissue powder to the Lysis Buffer as quickly as possible. Leaving the powder without the Lysis Buffer can result in degraded RNA.

- The homogenized tissue should be directly used for RNA purification and should not be stored.
- All homogenized material must be thoroughly mixed with the Lysis Buffer and should not be allowed to dry on the walls of the tube (this can cause degradation of RNA).

b) Disruption and homogenization using homogenizer:
Homogenize the tissue according manufacturer recommendations.

2. Add 40 µL of **MagJET Magnetic Beads** resuspended well by vortexing and 400 µL of **ethanol** (96-100%) to the lysate. Mix by vortexing for 5 minutes to obtain a uniform suspension. Spin briefly to collect droplets. Place the sample in the magnetic rack for 1 minute or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack remove and discard the supernatant carefully by using a pipette. Make sure that all of the supernatant is removed.
3. Add 200 µL **1X Reaction Buffer with $MgCl_2$ for DNase I and 5 µL DNase I.** Mix gently. Spin briefly to collect droplets. Incubate the tubes in the thermomixer at 37°C, 700 - 900 rpm, for 15 minutes.
4. Spin briefly to collect droplets. Add 200 µL **ethanol** (96-100%) to the tube. Vortex briefly, then mix by inverting the tube for 5 minutes. Spin briefly. Place the sample in the magnetic rack for 1 minute, or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack remove and discard the supernatant carefully by using a pipette. Make sure that all of the supernatant is removed.
5. Add 700 µL of **Wash buffer 1** (supplemented with ethanol). Mix by vortexing for 1 minute. Spin briefly. Place the sample in the magnetic rack for 1 minute or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack remove and discard the supernatant carefully by using a pipette. Make sure that all wash solution is removed.
6. Add 700 µL of **Wash buffer 2** (supplemented with ethanol). Mix by vortexing for 1 minute. Spin briefly. Place the tube in the magnetic rack for 1 minute or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack remove and discard the supernatant carefully by using a pipette. Make sure that all wash solution is removed.
7. Repeat step 6.
8. Add 100 µL* of **nuclease-free water**. Mix thoroughly by vortexing. Spin briefly. Incubate tubes in a thermomixer at 60°C**, 700 - 900 rpm for 5 minutes. Spin briefly. Place the tube in the magnetic rack for 1 minute or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack, carefully transfer eluate (containing RNA) to a new, sterile microcentrifuge tube. Keep on ice for immediate use in downstream applications or store at -20°C -70°C.

* If more concentrated RNA is required the volume of the nuclease-free water, can be reduced to 50 µL. If less concentrated RNA is required the volume of the water can be increased up to 150 µL.

** Incubating at 60°C increases RNA yield. If 30% lower yield of RNA is acceptable, tubes can be incubated at **room temperature** in thermomixer (700 - 900 rpm) for 5 minutes.

Protocol F. Instructions for total RNA purification from bacterial culture (up to 10^9 cells)

Important Note

For RNA isolation bacteria cells should be harvested during the exponential phase of growth (OD_{600}=0.5-1). Do not use an overnight culture for RNA isolation.

Before starting:

- Supplement the required amount of Lysis Buffer with DTT or β-mercaptoethanol. Add 10 µL 2 M DTT or 10 µL 14.3 M β-mercaptoethanol to each 450 µL volume of Lysis Buffer required.
- Supplement the required amount of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA) with lysozyme (not included) to final concentration of 0.4 mg/mL.

Note. When using the MagJET RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Reconstitute DNase I (lyophilized) in DNase I Reconstitution Buffer as described on page 5.
- Prepare 1X Reaction Buffer with $MgCl_2$ for DNase I as described on page 5.
- Transfer the **Tissue_RNA_Flex** protocol file to the KingFisher Flex or **Tissue_RNA_Duo** protocol file to the KingFisher Duo instrument before first use. 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. The protocol files for MagJET RNA Kit can be found on product web page on www.thermoscientific.com/onebio.

1	Transfer up to 1.5 mL of bacteria culture (up to 1 x 10^9 cells) to a 1.5 mL microcentrifuge tube (not provided). Collect cells by centrifugation for 2 min at ≥12,000 × g. Carefully remove the supernatant, leaving the pellet as dry as possible.
2	Resuspend the pellet in 100 µL of freshly prepared TE buffer supplemented with lysozyme (0.4 mg/mL final concentration). Invert the tube several times to mix.
3	Incubate the resuspended cells for 5 min at 15-25°C.
4	Add 450 µL of **Lysis Buffer** supplemented with **DTT or β-mercaptoehtanol**. Mix thoroughly by vortexing for about 15 s until a homogeneous mixture is obtained. Spin down the tube to collect all the drops from the walls of the tube.Transfer 450 µL of the prepared lysate to **RNA** or **Sample** plate (depends on instrument used for further purification).
5	For manual RNA purification, proceed to **Step 2** of the **Protocol E: Instructions for manual RNA purification from up to 2 × 10^6 cultured mammalian cells and up to 20 mg tissue** on page 15. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments, proceed to Step 4 of the **Protocol A: Instructions for total RNA purification from up to 2 × 10^6 cultured mammalian cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 9 or Step 4 of the **Protocol C: Instructions for total RNA purification from up to 2 × 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 13**.**

Protocol G. Instructions for total RNA purification from yeast culture (up to 10^8 cells)

Important Note:
For RNA isolation yeast cells should be harvested at the exponential phase of growth (OD_{600}=0.5-1). Do not use an overnight culture for RNA isolation. For cell disruption using enzymatic lysis (described below) use only freshly harvested cells.

Before starting:

- Prepare Yeast lysis buffer: 1 M sorbitol, 0.1 M EDTA, pH 7.4. Just prior to use add 0.1% β-mercaptoethanol and 5 mg/mL of Zymolyase 20T.

Note. When using the MagJET RNA Kit for the first time:

- Prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Reconstitute DNase I (lyophilized) in DNase I Reconstitution Buffer as described on page 5.
- Prepare 1X Reaction Buffer with $MgCl_2$ for DNase I as described on page 5.
- Transfer the Tissue_RNA_Flex protocol file to the KingFisher Flex or Tissue_RNA_Duo protocol file to the KingFisher Duo instrument before first use. 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. The protocol files for MagJET RNA Kit can be found on product web page on www.thermoscientific.com/onebio

1	Transfer the yeast cell culture (up to 1 x 10^8 yeast cells) to a 1.5 mL microcentrifuge tube (not provided). Collect the cells by centrifugation for 2 min at ≥12,000 x g. Discard the supernatant.
2	Resuspend the cell pellet in 100 µL of Yeast lysis buffer.
3	Incubate at 30°C for 30 min.
4	Add 450 µL of **Lysis Buffer**. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Spin down the tube to collect all the drops from the walls of the tube. Transfer 450 µL of the prepared lysate to **RNA** or **Sample** plate (depends on instrument used for further purification).
5	For manual RNA purification, proceed to Step 2 of the **Protocol E. Instructions for manual RNA purification from up to 2 × 10^6 cultured mammalian cells and up to 20 mg tissue** on page 15. For automated purification using KingFisher Flex 96 or KingFisher Duo instruments, proceed to Step 4 of the **Protocol A: Instructions for total RNA purification from up to 2 × 10^6 cells using KingFisher Flex 96 and Microtiter deep well 96 plates** on page 9 or Step 4 of the **Protocol C: Instructions for total RNA purification from up to 2 × 10^6 cultured mammalian cells using KingFisher Duo and Microtiter deep well 96 plates** on page 13**.**

TROUBLESHOOTING

Problem	Possible cause and solution
Low RNA yield	**Too much starting material was used for lysate preparation** Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocol. **Starting material was not completely disrupted** Reduce the amount of starting material. Increase disruption time. **Incomplete re-suspension of magnetic particles** Fully resuspend the magnetic particles by vortexing before use. **Ethanol was not added to the lysate** Make sure that ethanol was added to the lysate before loading Sample or RNA plate into the KingFisher Flex or King Fisher Duo instrument. **Prolonged storage of the sample material** Prolonged storage of the sample material may reduce the total RNA yield. **Too small amount of nuclease free water in elution step** There should be an adequate volume of the nuclease free water to cover the magnetic beads completely during the elution step. Do not use less nuclease free water than indicated in the protocol. *Manual protocol only:* **Loss of magnetic beads during manual purification** Be careful not remove the magnetic beads during purification using manual protocol**.** **Not fully resuspended magnetic beads during binding step** Mix in the thermomixer at least 5 minutes (or longer if it's necessary) to obtain a uniform suspension. **Not fully dispersed magnetic beads during elution step** Make sure the magnetic beads are fully dispersed in nuclease-free water during elution step.
Degraded RNA	**Inappropriate handling of starting material** When purifying RNA from fresh samples, place samples in liquid nitrogen immediately after harvesting. Proceed to lysis and homogenization as quickly as possible. Ensure that samples are frozen in liquid nitrogen immediately after collection and stored at -70°C. Thawing of the samples should be avoided until addition of Lysis Buffer. **RNase contamination** To avoid RNase contamination, wear gloves during all procedures and change gloves frequently. Use sterile, disposable RNase-free pipette tips. Remove RNase contamination from non-disposable items and work surfaces.
Low purity	**Insufficient washing** Insufficient washing causes impurities in the Elution step with nuclease free water. Ensure that volumes of the Wash Buffer 1 and 2 are as indicated in the protocol. *Manual protocol only:* **Not fully removed Wash Buffer 1 or Wash Buffer 2 during Wash steps** Make sure that all wash solution is removed during wash steps.
Magnetic particles in the purified RNA	Carryover of the MagJET Magnetic Beads to the Elution step may affect the A_{260}/A_{280} ratio, however the magnetic beads in the eluted RNA will not affect downstream applications. To remove carryover magnetic particles place eluted sample in the magnetic rack once again. Carefully transfer eluate to a clean, sterile microcentrifuge tube. 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 into the nuclease free water.

SAFETY INFORMATION

Lysis Buffer for MagJET RNA Kit

Xn Harmful
Hazard-determining components of labeling: guanidinium thiocyanate
Risk phrases
22 Harmful if swallowed.
38 Irritating to skin.
41 Risk of serious damage to eyes.
52/53 Harmful to aquatic organisms. May cause long-term adverse effects in the aquatic environment.
Safety phrases
23 Do not breathe gas/fumes/vapour/spray.
26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
60 This material and its container must be disposed of as hazardous waste.
61 Avoid release to the environment. Refer to special instructions/safety data sheets.

Lysis Buffer for MagJET RNA Kit

Warning
Hazard statements:
H302 Harmful if swallowed.
H412 Harmful to aquatic life with long lasting effects.
Precautionary statements:
P273 Avoid release to the environment.
P264 Wash thoroughly after handling.
P270 Do not eat, drink or smoke when using this product.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330 Rinse mouth.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Wash Buffer 1 (conc.) for MagJET RNA Kit

Xn Harmful
Hazard-determining components of labeling: guanidinium chloride
Risk phrases
22 Harmful if swallowed.
36/38 Irritating to eyes and skin.
Safety phrases
3 Keep in a cool place.
23 Do not breathe gas/fumes/vapour/spray.
26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
36/37 Wear suitable protective clothing and gloves.
60 This material and its container must be disposed of as hazardous waste.

Wash Buffer 1 (conc.) for MagJET RNA Kit

Warning
Hazard statements:
H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
Precautionary statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P321 Specific treatment (see on this label).
P362 Take off contaminated clothing and wash before reuse.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

PRODUCT USE LIMITATION
This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.