ISOLATE II
RNA Mini Kit

Product Manual

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ISOLATE II RNA Mini Kit

1. KIT CONTENTS

COMPONENT	10 Preps	50 Preps	250 Preps
ISOLATE II Filters (violet)	10	50	250
ISOLATE II RNA Mini Columns (blue) & Collection Tubes	10	50	250
Collection Tubes (2 mL)	30	150	750
Collection Tubes (1.5 mL)	10	50	250
Lysis Buffer RLY*	10 mL	25 mL	125 mL
Wash Buffer RW1*	13 mL	13 mL	80 mL
Wash Buffer RW2† (concentrate)	6 mL	12 mL	3 x 25 mL
Membrane Desalting Buffer MEM*	10 mL	25 mL	125 mL
Reaction Buffer for DNase I RDN	7 mL	7 mL	30 mL
DNase I, RNase-free (lyophilized)	1 vial	1 vial	5 vials
Bench Protocol Sheet	1	1	1

** Contains a guanidine salt. Not compatible with disinfectants containing bleach or acidic solutions. See safety information in section 4.*
† Before use, add indicated volume of 96-100% ethanol and mark wash buffer bottle label.

2. DESCRIPTION

The ISOLATE II RNA Mini Kit is a simple, reliable and fast method for isolation of high-quality total cellular RNA from a wide variety of sources including cells and tissue from human/animal sources, bacteria, yeast and biological liquid samples.

Biological samples are first lysed and homogenized in the presence of guanidinium thiocyanate, a chaotropic salt which immediately deactivates endogenous RNases to ensure purification of intact RNA. After homogenization, ethanol is added to the sample. The sample is then processed through a spin column containing a silica membrane to which the RNA binds. Genomic DNA contamination is removed by an on-column DNase I digestion during the preparation (RNase-free DNase I is supplied). Any impurities such as salts, metabolites and cellular components are effectively removed by simple washing steps with two different buffers. High-quality purified total RNA is then eluted in RNase-free water (supplied).

The purified total RNA eluted using the ISOLATE II RNA Mini Kit is suitable for use in a variety of applications, including:

- Real-time PCR
- Reverse-transcriptase PCR (RT-PCR)
- cDNA synthesis
- Next generation sequencing
- Northern, dot and slot blotting
- Nuclease protection assays
- RNA amplification for microarray analysis
- cDNA library preparation after poly(A)+ selection

Please read this manual carefully to familiarise yourself with the ISOLATE II RNA Mini protocol before starting (also available on www.bioline.com). More experienced users can refer to the bench-top protocol for quick referencing during the procedure.

3. STORAGE

Store lyophilized DNase I (RNase-free) at 4°C on arrival (stable for up to 1 year). Reconstituted DNase I working solution is stable for 6 months at -20°C. All other kit components should be stored at room temperature (18–25°C) and are stable for up to 1 year. Storage at lower temperatures may cause precipitation of salts.

4. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Buffers RLY, RW1 and MEM contain guanidine thiocyanate. This chemical is harmful when in contact with skin, inhaled or ingested.

CAUTION: Do not add bleach directly to solutions or sample preparation waste containing guanidine salts. Reactive compounds and toxic gases can form. Clean with a suitable laboratory detergent and water if liquid from these buffers are spilt.

For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

The ISOLATE II RNA Mini Kit is specially designed for the rapid and efficient isolation of extremely pure total RNA. The kit is compatible with cultured cells, tissue, yeast, bacteria and biological liquid (cell-free) samples. The preparation time is approx. 30 min for 6 preps. The isolated RNA is of high-purity (A_{260}/A_{280} ratio: >1.9) and high-integrity (RIN >9) for fresh, high-quality samples (see below).

ISOLATE II RNA MINI SPIN COLUMN SPECIFICATIONS	
Max. binding capacity	200 µg RNA
RNA size distribution	>200 nucleotides
A_{260}/A_{280} ratio*	1.9–2.1
Typical RIN (RNA integrity number) †	>9
Elution volume	40–120 µL
Max. amount of starting material	
Cultured cells (human/other mammalian)	5×10^6
Tissue (human/other mammalian)	30 mg
Yeast cells	1×10^8
Bacterial cells	1×10^9

** Typically, the A_{260}/A_{280} ratio exceeds 1.9, indicating excellent RNA purity.*
† Agilent 2100 Bioanalyzer (RNA 6000 assay). RNA integrity is highly dependent on sample quality.

Typical RNA Yield

It is important to use the correct amount of starting material in order to obtain optimal RNA yield and purity. Several factors determine the maximum amount that can be used including: sample type and RNA content, volume of lysis buffer needed for efficient lysis and binding capacity of the ISOLATE II RNA Mini spin column. Table 1 shows expected RNA yields from different sources. Depending on sample type, the average yield is between 5–70 µg total RNA.

TABLE 1: TYPICAL YIELDS OF TOTAL RNA WITH ISOLATE II RNA MINI KIT

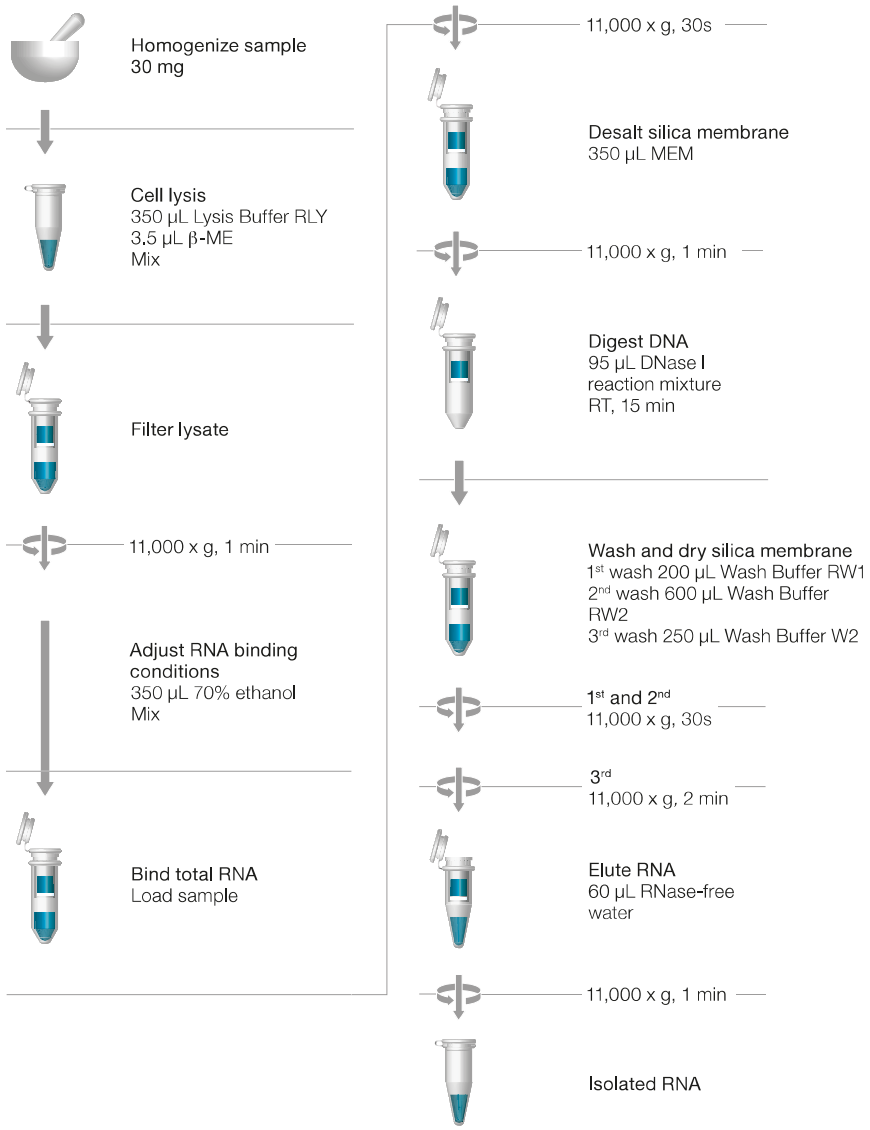
Sample Type	Source	Total RNA Yield* (µg)
Mammalian cell cultures ($1x10^6$ cells)	NIH/3T3	10
	HeLa	15
Prokaryotic cell cultures ($1x10^9$ cells)	Bacteria	70
Mouse/rat tissues (10 mg)	Liver	50
	Kidney	25
	Spleen	40
	Brain	5
	Thymus	45
Yeast ($1x10^7$ cells)	*S. cerevisiae*	25

** The standard protocol purifies up to 70 µg of total RNA per ISOLATE II RNA Mini Column from up to 5 x 10^6 eukaryotic cultured cells or 30 mg of tissue. Yields can vary depending on factors such as species, stage of development and culture conditions.*

The following components are also included in the kit:

- ISOLATE II Filters for homogenization and reduction of lysate viscosity.
- DNase I (RNase-free) for eliminating genomic DNA contamination by on-column digestion or by digestion in solution (for the most sensitive applications).

Total RNA Isolation



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

When working with chemicals, always wear a suitable lab coat, protective goggles and disposable gloves. Please consult the relevant MSDS from the product supplier for further information.

- ß-mercaptoethanol (ß-ME)* (for Lysis Buffer RLY)
- 70% ethanol† (to adjust RNA binding conditions)
- 96–100% ethanol† (for Wash Buffer RW2)
- Equipment for sample disruption and homogenization (see section 7.2). One or more of the following are required depending on chosen method:
  - PBS and trypsin
  - Needle and syringe (both RNase-free)
  - Mortar and pestle
  - Rotor-stator or bead-mill homogenizer
  - Liquid nitrogen
- Microcentrifuge tubes (1.5 mL)
- Sterile RNase-free tips
- Pipettes
- Microcentrifuge (capable of 11,000 x g)

** Dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP) are also suitable reducing agents instead of ß-ME*

† Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone. Make 70% ethanol up with nuclease-free molecular biology grade water.

7. IMPORTANT NOTES

The ISOLATE II RNA Mini purification procedures can be performed at room temperature. Handle the eluted RNA carefully to avoid contamination by RNases, often found on labware, fingerprints and dust. For optimal RNA stability, keep RNA frozen at -20°C for short-term or -80°C for long-term storage. When working with RNA samples in downstream applications, keep the RNA solution on ice.

See hints and tips on working with RNA at www.bioline.com/isolate.

7.1 HANDLING AND STORING STARTING MATERIALS

RNA is not protected against digestion until the sample is flash frozen or disrupted in the presence of RNase inhibiting or denaturing reagents. Samples should be flash frozen in liquid nitrogen immediately and stored at -80°C or processed as soon as possible. Following disruption and homogenization in Lysis Buffer RLY, samples can be kept at -80°C for up to one year, at 4°C for up to 24 hours or up to several hours at room temperature. Frozen

samples are stable for up to 6 months. Frozen samples in Lysis Buffer RLY should be thawed slowly before starting the isolation of total RNA. Animal and yeast cells can be pelleted and stored at -80°C until processing. For RNA isolation of yeast cells with enzymatic lysis, only fresh samples can be used.

7.2 DISRUPTING AND HOMOGENIZING STARTING MATERIALS

For all RNA purification procedures, efficient disruption and homogenization of starting material is essential. To release all RNA contained in a sample, total disruption of cell walls, plasma membranes and organelles must occur. Incomplete disruption results in reduced RNA yields. Homogenization reduces lysate viscosity following disruption and facilitates efficient binding of RNA to the column membrane. Incomplete homogenization results in inefficient binding of RNA to the membrane and therefore reduced RNA yields.

Cells grown in suspension

Centrifuge an appropriate number of cells and remove all supernatant by aspiration. Directly lyse by adding Lysis Buffer RLY. Refer to step 2 of the standard protocol (see section 8.1).

Cells grown in a monolayer

Remove the cell culture medium completely. Incomplete removal of the medium will inhibit lysis of the cells and compromise the efficiency of RNA isolation. Add Lysis Buffer RLY immediately to the cell culture plate. Refer to step 2 of the standard protocol (see section 8.1)

Trypsinzation of adherent cells

To trypsinize adherent growing cells, first aspirate the cell culture medium. Add an equal volume of PBS to wash the cells and aspirate excess liquid. Add 0.1–0.3% trypsin in PBS to the washed cells. Incubate until cells are detached. Add culture medium and transfer cells to an appropriate tube (not supplied). Pellet cells by centrifugation (5 min at 300 x g). Remove supernatant and add Lysis Buffer RLY to the cell pellet.

Disruption using a mortar and pestle

An RNase-free mortar and pestle can be used in combination with liquid nitrogen to disrupt and lyse frozen or fibrous tissue samples, which are often solid. Grind the frozen tissue into a fine powder and add liquid nitrogen as necessary. It is important to ensure the sample does not thaw during or after grinding. Then transfer tissue powder into a liquid nitrogen cooled tube and allow liquid nitrogen to evaporate. Add Lysis Buffer RLY with reducing agent to powdered tissue and mix immediately. Homogenize sample with an ISOLATE II Filter (supplied) or by passing 5-10 times through a nuclease-free 20 gauge (0.9 mm) syringe needle.

Disruption and homogenization using a rotor-stator homogenizer

Rotor-stator type tissue homogenizers can homogenize, disrupt and emulsify animal tissue samples in the presence of lysis buffer in seconds to minutes. Homogenization time depends on sample size and toughness. The spinning rotor disrupts and homogenizes the sample simultaneously by turbulence and mechanical shearing. Foaming can be minimized by keeping the rotor tip submerged. Select a suitably sized homogenizer: 5–7 mm diameter rotors can be used for homogenization in microcentrifuge tubes.

Disruption and homogenization by enzymatic digest (bacteria/yeast)

For effective yeast RNA isolation, zymolase/lyticase treatment is required to degrade polysaccharides and proteins in the cell wall prior to cell lysis (section 8.2). For bacterial RNA isolation, lysozyme treatment is necessary to ensure efficient cell lysis and RNA release from the cells (section 8.3). For preparation of RNA from microorganisms with difficult to disrupt cell walls e.g. certain Gram positive bacteria, optimizing treatment conditions with lytic enzymes or culture conditions may be required. Following lysis, perform homogenization with an ISOLATE II RNA Filter with a syringe and needle.

7.3 BUFFER PREPARATION AND PARAMETERS

Ensure 70% ethanol and reducing agent (ß-ME, DTT, or TCEP) is available.
For all protocols prepare the following:

Preparing DNase I (RNase-free) stock solution

Reconstitute lyophilized DNase I in RNase-free water (supplied): Add 230 µL water for the 10 prep kit, 540 µL for the 50 prep kit and 540 µL x 5 for the 250 prep kit. Incubate for 1 min at room temperature, then mix by gently swirling.

***Important Note**: Do not vortex the reconstituted DNase I as the enzyme is particularly sensitive to mechanical denaturation. Dispense into aliquots to avoid excessive freeze-thawing. Do not freeze/thaw aliquots more than three times. Store aliquots at -20°C. The frozen working solution is stable for 6 months.*

Preparing Wash Buffer RW2 with ethanol

Add 96–100% ethanol to Wash Buffer RW2 Concentrate: Add 20 mL ethanol for the 10 prep kit, 50 mL for the 50 prep kit and 100 mL x 3 for the 250 prep kit.

***Important Note**: Mark the label of the bottle to indicate ethanol was added. Store Wash Buffer RW2 at room temperature (18–25°C) for up to one year.*

Amount of Lysis Buffer RLY needed

Before beginning lysis and homogenization steps, a fresh aliquot of Lysis Buffer RLY with denaturing agent should be prepared. The kit can be used for preparing RNA from different starting sample materials and amounts. Use Table 2 to determine the correct volume of Lysis Buffer RLY and ethanol required. Insufficient lysis or washing will result in sub-optimal

RNA yield and purity. An extra loading step is required if 600 µL Lysis Buffer RLY and 600 µL ethanol are used. The sample should be loaded onto the column in two successive centrifugation steps.

TABLE 2: LYSIS BUFFER AND ETHANOL VOLUMES FOR DIFFERENT STARTING AMOUNTS OF MATERIAL

Sample	Amount	Volume	
		Lysis Buffer RLY (Step 2)	Ethanol (Step 4)
Cultured human or mammalian cells	<5 x 10^6	350 µL	350 µL
Human or mammalian tissue	<20 mg	350 µL	350 µL
	20 mg–30 mg*	600 µL	600 µL
Tissue stored in RNA*later*®	<20 mg	350 µL	350 µL
	20 mg–30 mg*	600 µL	600 µL
Difficult to lyse samples	<5 x 10^7	600 µL	600 µL

** Extra volume of Lysis Buffer RLY is required for protocols using 600 µL instead of 350 µL of buffer.*

Elution parameters

Elute RNA using RNase-free water (included). The standard elution protocol can be modified for different applications. To achieve high yield, perform two successive elution steps with an elution volume described in the individual protocol (90–100% recovery rate). For both high-yield and high-concentration, elute with the standard elution volume. Then re-apply eluate onto the column for re-elution.

Always place eluted RNA on ice immediately to prevent degradation by RNases. For short-term storage freeze at -20°C, for long-term storage freeze at -80°C.

7.4 ELIMINATING GENOMIC DNA CONTAMINATION

Genomic DNA contamination is efficiently removed by on-column digestion with DNase I (supplied). Residual genomic DNA may however be detected in very sensitive applications e.g. probe-based real-time PCR. A DNase I digest in the eluate can be performed to remove even traces of contaminating DNA (see section 8.7).

8. PROTOCOLS

8.1 PURIFYING TOTAL RNA FROM CULTURED CELLS AND TISSUE

Before you start:

- Ensure Wash Buffer RW2 and DNase I are prepared (see section 7.3)

Additional reagents/components required:

- ß-ME or DTT or TCEP (see section 6)

1 **Sample homogenization**

Up to 5 x 10^6 eukaryotic cultured cells can be collected by centrifugation and directly lysed by adding Lysis Buffer RLY.

Disrupt up to 30 mg of tissue. For sample amounts see Table 1; for homogenization methods see section 7.2.

2 **Cell lysis**

Add 350 µL Lysis Buffer RLY and 3.5 µL ß-ME to the cell pellet or to ground tissue and vortex vigorously.

For appropriate sample amounts and Lysis Buffer volumes see Table 1 and 7.3 respectively.

Note: DTT or TCEP may be used in place of ß-ME. Use a final concentration of 10–20 mM DTT or TCEP in Lysis Buffer RLY.

3 **Filter lysate**

Place ISOLATE II Filter (violet) in a Collection Tube (2 mL), load the lysate and centrifuge for 1 min at 11,000 x g. This step helps reduce viscosity and clears the lysate.

If there is a visible pellet formed, depending on sample amounts and properties, transfer supernatant avoiding any pellet to a new 1.5 mL microcentrifuge tube (not supplied). Alternatively, pass lysate 5-10 times through a nuclease-free 20 gauge (0.9 mm) needle and syringe.

***Important Note**: To process higher amounts of cells (>1x10^6) or tissue (>10 mg), the lysate should first be homogenized with a nuclease-free 20 gauge (0.9 mm) needle and syringe, then filtered through an ISOLATE II Filter.*

4 **Adjust RNA binding conditions**

Discard ISOLATE II Filter and add 350 µL ethanol (70%) to the homogenized lysate. Mix by pipetting up and down 5 times.

Alternatively, transfer the flow-through into a new 1.5 mL microcentrifuge tube (not supplied), add 350 µL ethanol (70%) and mix by vortexing (2 x 5s).

Note: After addition of ethanol a stringy precipitate may become visible. This will not affect the RNA isolation. Break apart any precipitate by mixing. Do not centrifuge the ethanolic lysate before loading it onto the column to prevent pelleting the precipitate.

5 **Bind RNA**

For each preparation place one ISOLATE II RNA Mini Column (blue) in a Collection Tube (2 mL). Pipette lysate up and down 2–3 times and load lysate onto the column. Ensure all of the lysate is loaded on the column. Centrifuge for 30s at 11,000 x g. Place column in a new Collection Tube (2 mL).

Note: Maximal loading capacity of ISOLATE II RNA Mini Column is 750 µL. Repeat procedure if larger volumes are to be processed.

6 **Desalt silica membrane**

Add 350 µL Membrane Desalting Buffer (MEM) and centrifuge at 11,000 x g for 1 min to dry the membrane.

Note: Salt reduces DNase I activity in step 7. If column outlet accidentally touches the flow-through, discard the flow-through and centrifuge again for 30s at 11,000 x g.

7 **Digest DNA**

Prepare a DNase I reaction mixture (see section 7.3) in a sterile 1.5 mL microcentrifuge tube (not supplied). For each isolation, add 10 µL reconstituted DNase I (also see section 7.3) to 90 µL Reaction Buffer for DNase I (RDN). Mix by gently flicking the tube. Apply 95 µL DNase I reaction mixture directly onto center of silica membrane. Incubate at room temperature for 15 min.

8 **Wash and dry silica membrane**

1st Wash

- Add 200 µL Wash Buffer RW1 to the ISOLATE II RNA Mini Column. Centrifuge for 30s at 11,000 x g. Place the column into a new Collection Tube (2 mL).

Note: Wash Buffer RW1 will inactivate the DNase I.

2nd Wash

- Add 600 µL Wash Buffer RW2 to the ISOLATE II RNA Mini Column. Centrifuge for 30s at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.

Note: Ensure residual buffer from previous steps is washed away with Wash Buffer RW2, especially if lysate has contacted the inner rim of the column during loading of the lysate onto the column. To efficiently wash the inner rim, flush it with Wash Buffer RW2.

3rd Wash

- Add 250 µL Wash Buffer RW2 to the ISOLATE II RNA Mini Column. Centrifuge for 2 min at 11,000 x g to dry the membrane completely. Place the column into a nuclease-free 1.5 mL Collection Tube (supplied).

If for any reason, the liquid level in the Collection Tube during washes 1, 2 or 3 has reached the ISOLATE II RNA Column after centrifugation, discard flow-through and centrifuge again.

9 **Elute RNA**

Elute the RNA with 60 µL RNase-free water (supplied) and centrifuge at 11,000 x g for 1 min.

If more concentrated RNA is required, perform elution with 40 µL. However, smaller volumes than this may result in reduced RNA yield.
For further alternative elution procedures, see section 7.3.

8.2 PURIFYING TOTAL RNA FROM YEAST

This protocol is designed for preparing total RNA from yeast (up to 5 x 10^8 cells). The protocol includes two different options for homogenizing yeast cells: enzymatic digestion and mechanical disruption. In general, both function equally well. An enzymatic digest requires no additional equipment but is only recommended for freshly harvested cells. Mechanical disruption is well-suited for time-course experiments where enzymatic digests are not practical. Mechanical disruption can be performed on yeast cell pellets previously stored at -80°C for several months.

Before you start:

- Ensure Wash Buffer RW2 and DNase I are prepared (section 7.3)

Additional reagents/components required:

- ß-ME or DTT or TCEP
- Sorbitol and lyticase (or zymolase) for homogenization by enzymatic digestion or
- Swing-mill and glass beads for homogenization by mechanical disruption may also be used.

1 Sample Homogenization

1.1 Enzymatic Digestion

Note: Homogenization by enzymatic digest is only recommended for freshly harvested cells and is not recommended for time-course experiments.

- Harvest 2–5 mL of YPD culture (5000 x g, 10 min).
- Resuspend pellet in an appropriate amount of freshly prepared sorbitol/lyticase buffer (50–100U lyticase or zymolase in 1 M sorbitol/100 mM EDTA) (not supplied).
- Incubate at 30°C for 30 min.
- Pellet resulting spheroplasts by centrifugation (1000 x g, 10 min).
- Carefully discard supernatant.

Note: Incubation time and lyticase/zymolase concentration may need optimization, depending on yeast cell type and sample size.

Proceed to step 2 (see next page).

or

1.2 **Mechanical Disruption**

- Harvest 2–5 mL of YPD culture (5000 x g, 10 min) and wash with ice-cold water.
- Resuspend the cell pellet in a mixture of 350 µL Lysis Buffer RLY and 3.5 µL ß-ME.
- Add glass beads (e.g. 300 mg glass beads, 425–600µm, Sigma #68772 (not supplied)).
- Shake samples in a swing-mill (30 Hz; 15 min).

Note: DTT or TCEP may be used in place of ß-ME. Use a final concentration of 10–20 mM DTT or TCEP within Lysis Buffer RLY.

Skip step 2 and proceed to step 3 (see below).

2 **Cell lysis**

Add 350 µL Lysis Buffer RLY and 3.5 µL ß-ME and vortex vigorously to lyse spheroplasts.

For appropriate sample and lysis buffer amounts see sections 5 and 7.3 respectively.

Note: DTT or TCEP may be used in place of ß-ME. Use a final concentration of 10–20 mM DTT or TCEP within the Lysis Buffer RLY.

3 **Filter lysate**

Place ISOLATE II Filter in Collection Tube (2 mL), apply lysate, and centrifuge for 1 min at 11,000 x g. This step reduces solution turbidity and viscosity.

If a visible pellet forms, depending on sample amount and yeast type, transfer supernatant without any pellet to a new 1.5 mL microcentrifuge tube (not supplied).

Alternatively, pass lysate ≥ 5 times through a nuclease-free 20 gauge (0.9 mm) needle and syringe.

4 **Adjust RNA binding conditions**

Add 350 µL of ethanol (70%) to the lysate and mix by vortexing.

Proceed with step 5 of the ISOLATE II RNA Mini standard protocol (see section 8.1).

8.3 PURIFYING TOTAL RNA FROM BACTERIA

This protocol is designed for preparing total RNA from bacteria (up to 10^9 cells).

Before you start:

- Ensure Wash Buffer RW2 and DNase I are prepared (section 7.3)

Additional reagents required:

- Lysozyme
- TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8)

1 **Sample homogenization**

Resuspend the bacterial cell pellet (Gram-negative strains) in 100 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8) containing 1 mg/mL lysozyme by vigorous vortexing. Incubate at 37°C for 10 min.

For Gram-positive bacteria, resuspend cells in 100 µL TE buffer containing 2 mg/mL lysozyme. It may be necessary to optimize incubation time and lysozyme concentration, depending on the bacterial strain.

Note: Due to the much higher concentration of nucleic acid in bacteria compared with eukaryotic material, it may be necessary to use a lower number of cells.

2 **Cell lysis**

Add 350 µL Lysis Buffer RLY and 3.5 µL ß-ME to the suspension and vortex vigorously. For appropriate sample and lysis buffer amounts see Table 1 and 7.3, respectively.

Note: DTT or TCEP may be used in place of ß-ME. Use a final concentration of 10–20 mM DTT or TCEP within the Lysis Buffer RLY.

3 **Filter lysate**

Reduce viscosity and turbidity of the solution by filtration through an ISOLATE II Filter (violet). Place ISOLATE II Filter in Collection Tube (2 mL), apply mixture and centrifuge for 1 min at 11,000 x g.

If a visible pellet forms, depending on cell numbers and bacterial strain, transfer supernatant avoiding any pellet to a new 1.5 mL microcentrifuge tube (not supplied). Alternatively, the lysate may be passed ≥5 times through a nuclease-free 20 gauge (0.9 mm) needle and syringe.

4 **Adjust RNA binding conditions**

Add 350 µL of ethanol (70%) to the lysate and mix by vortexing.

Proceed with step 5 of the ISOLATE II RNA Mini standard protocol (see section 8.1).

8.4 PURIFYING TOTAL RNA FROM BIOLOGICAL LIQUIDS

This protocol is designed for purifying total RNA from biological liquids such as serum or culture medium.

Before you start:

- Ensure Wash Buffer RW2 and DNase I are prepared (see section 7.3).

1 **Sample homogenization**

Not required.

2 **Cell lysis**

Add 350 µL Lysis Buffer RLY and 3.5 µL ß-ME to 100 µL of sample and vortex vigorously.

For appropriate sample and lysis buffer amounts see sections 5 and 7.3 respectively.

Note: DTT or TCEP may be used in place of ß-ME. Use a final concentration of 10–20 mM DTT or TCEP within the Lysis Buffer RLY.

3 **Filter lysate**

Not required.

4 **Adjust RNA binding conditions**

Add 350 µL of ethanol (70%) to the lysate and mix by vortexing.

Proceed with step 5 of the ISOLATE II RNA Mini standard protocol (section 8.1).

8.5 PURIFYING TOTAL RNA FROM PARAFFIN EMBEDDED TISSUE

This protocol is designed for purification of total RNA from paraffin-embedded tissue sections. Paraffin is first dissolved in xylene (not supplied). After precipitation of the sample and removal of the supernatant, residual xylene is removed by washing with ethanol.

Before you start:

- Ensure Wash Buffer RW2 and DNase I are prepared (section 7.3).

Additional reagent required:

- Xylene (to deparaffinize tissue sections)

1 **Deparaffinization of tissue**

- Transfer 10 mg of finely sliced tissue into a 1.5 mL microcentrifuge tube (not supplied).

A Add 300 µL xylene. Incubate at room temperature for 5 min with constant mixing.

B Centrifuge at maximum speed (11,000 x g) for 3 min to pellet the tissue. Discard the xylene.

C Repeat steps 1A and 1B twice, to give three xylene washes in total.

2 **Wash with ethanol**

A Add 300 µL of 96% ethanol to the tube and incubate at room temperature for 5 min with constant mixing.

B Centrifuge at maximum speed (11,000 x g) for 3 min to pellet the tissue. Discard the ethanol.

C Repeat steps 2A and 2B, to give two ethanol washes in total.

Continue with step 1 of the ISOLATE II RNA Mini standard protocol (section 8.1).

8.6 PURIFYING TOTAL RNA FROM RNA*later*® TREATED SAMPLES

Before you start:

- Ensure Wash Buffer RW2 and DNase I are prepared (see section 7.3).

1 **Sample preparation**

Remove RNA*later*® solution. Cut an appropriate amount of tissue.

2 **Cell lysis**

Add 350 µL Lysis Buffer RLY and 3.5 µL ß-ME to sample. Disrupt sample material with an appropriate method e.g. rotor-stator homogenizer. See section 7.2 for homogenization methods.

Proceed with step 3 of the ISOLATE II RNA Mini standard protocol (section 8.1)

8.7 DNASE I TREATMENT OF PURIFIED RNA IN SOLUTION

The on-column DNase I digestion results in minimal residual DNA undetectable in most downstream applications. For the most sensitive applications, DNA digestion in solution is recommended to eliminate even traces of contaminating DNA. Stringent RNase control as well as RNA repurification to remove buffer, salts, DNase I and digested DNA are needed.

1 **Digest DNA (Reaction setup)**
Add 6 µL Reaction Buffer for DNase I (RDN) and 0.6 µL DNase I to 60 µL eluted RNA. (Alternatively, premix 100 µL RDN and 10 µL DNase I and add 1/10th volume to one volume of RNA eluate).
Gently swirl tube to mix solution. Gently spin down (approx. 1s at 1,000 x g) to collect all the solution at the bottom of the tube.

2 **Sample incubation**
Incubate for 10 min at 37°C.

3 **RNA repurification**
Repurify RNA with a suitable RNA cleanup procedure, e.g. ISOLATE II RNA Micro Clean-Up Kit or by ethanol precipitation.
Ethanol precipitation step
- Add 1/10th volume of sodium acetate (3 M, pH 5.2)
- Add 2.5-3 x volume of 96–100% ethanol to one volume of sample. Mix thoroughly.
- Precipitate for one hour or overnight at -20°C.

Note: Choose longer incubation times if the sample has a low RNA concentration. Shorter incubation times are sufficient for high RNA concentrations.
- Centrifuge at maximum speed for 10 min.
- Wash RNA pellet with ice-cold 70% ethanol.
- Dry RNA pellet and resuspend RNA in RNase-free water.

8.8 CLEAN-UP OF RNA FROM REACTION MIXTURES

Before you start:
- Ensure Wash Buffer RW2 is prepared (see section 7.3).

1 **Sample preparation**
Bring volume up to 100 µL with RNase-free water for RNA samples <100 µL.
For RNA samples with volumes between 100-200 µL, bring up to a uniform volume (e.g. 200 µL) with RNase-free water.

2 **Prepare lysis-binding buffer premix**
Prepare a Lysis Buffer RLY-ethanol premix at 1:1 ratio.
For each 100 µL RNA sample, mix 300 µL Lysis Buffer RLY and 300 µL ethanol (96–100%).
A master-premix is recommended for multiple samples (e.g. 2 mL Lysis Buffer RLY + 2 mL 98% ethanol for approx. 6 preparations).

3 **Filter lysate**
Not required.

4 **Adjust RNA binding conditions**

To 100 µL of RNA sample, add 0.6 mL of the Lysis Buffer RLY-ethanol premix. Mix by vortexing.

To process 200 µL of RNA sample, add 1.2 mL of the Lysis Buffer RLY-ethanol premix. The ISOLATE II RNA Mini Column has a maximum loading capacity 750 µL; repeat procedure if larger volumes are required.

Note: After addition of ethanol a stringy precipitate may appear. This does not affect the RNA isolation. Be sure to mix thoroughly and apply sample as a homogeneous solution onto the column.

Proceed with step 5 of the ISOLATE II RNA Mini standard protocol (section 8.1)

9. TROUBLESHOOTING GUIDE

CLOGGED SPIN COLUMN	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Insufficient disruption or homogenization	Reduce starting material or use a larger volume of Lysis Buffer RLY. Ensure thorough disruption and use ISOLATE II Filters for homogenization of disrupted starting material.
Insufficient centrifugation	Increase centrifugation speed and time.
LOW RNA YIELD OR QUALITY	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Insufficient disruption or homogenization	Reduce starting material or use larger volume of Lysis Buffer RLY. Ensure thorough disruption and use ISOLATE II Filters for homogenization of disrupted starting material.
Sample material degraded	Store sample material properly. Use fresh material whenever possible; if not, flash-freeze sample in liquid nitrogen. Always keep samples at -80˚C. Always add Lysis Buffer before thawing sample. Disrupt samples in liquid nitrogen and ensure tubes are kept chilled.
Incomplete elution	Incubate sample in Elution Tube with RNase-free water for up to 5 minutes and repeat elution step.
Reagents not properly prepared	Add suitable reducing agent to Lysis Buffer RLY. Add 96-100% ethanol to Wash Buffer RW2 concentrate. Ethanol is required to create effective binding conditions for RNA to the silica membrane. Add RNase-free water to DNase I.

LOW RNA CONCENTRATION	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
High elution volume	Elute RNA with a lower volume.
Inappropriate handling and storing of starting material	Ensure proper handling and storage of samples. Ensure that all steps are followed quickly.
LOW A_{260}/A_{230} RATIO	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Guanidinium thiocyanate carryover	Carefully load lysate to ISOLATE II RNA Mini column, avoid contamination between column and lid. Ensure residual Wash Buffer RW1 is cleaned with Wash Buffer RW2. Apply Wash Buffer RW2 to inner rim of column.
RNA DEGRADED	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
RNase contamination	Ensure an RNase free working environment (see online hints and tips at www.bioline.com/isolate). Discard any solutions contaminated with RNase during use.
DNA CONTAMINATION	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Incorrect lysis	Check protocol has been followed correctly.
Too much starting material	Reduce amount of starting material.
DNase I inactive	Reconstitute lyophilized DNase I and store as recommended.
Oversensitive DNA detection	Use intron spanning primers if possible Use larger PCR amplicons (DNA detection probability increases with smaller PCR amplicons). Use DNase I digestion in solution protocol.

A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please email us at tech@meridianlifescience.com

B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II RNA Mini Kit	10 Preps	BIO-52071
ISOLATE II RNA Mini Kit	50 Preps	BIO-52072
ISOLATE II RNA Mini Kit	250 Preps	BIO-52073

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II RNA Micro Kit	10 Preps	BIO-52074
ISOLATE II RNA Micro Kit	50 Preps	BIO-52075
ISOLATE II RNA Plant Kit	10 Preps	BIO-52076
ISOLATE II RNA Plant Kit	50 Preps	BIO-52077
RiboSafe RNase Inhibitor	2,500 Units	BIO-65027

D. PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace free of charge any product that does not conform to the specifications. This warranty limits Bioline's liability only to the replacement of the product.

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