



GENETIX BRAND

HANDBOOK



Total RNA Isolation

<input type="checkbox"/> RNASure® Mini Kit	NP-84105	50 Preps
<input type="checkbox"/> RNASure® Mini Kit	NP-84107	250 Preps



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COMPONENTS OF THE KIT

	NP-84105	NP-84107
Number of Preps	50 preps	250 preps
Lysis Buffer LBA1	40ml	200ml
Wash Buffer (concentrated) WBA2	40ml	200ml
Wash Buffer (concentrated) WBA3	23ml	100ml
RNase free Water	30ml	125ml
Proteinase K	600µl	5 x 600µl
RNASure Mini Columns (with Collection Tubes)	50	250
Collection Tubes (2 mL)	50	250
Collection Tubes (1.5 mL)	50	250

INTRODUCTION

Principle and Procedure

Samples are lysed and homogenized in Lysis Buffer LBA1, which contains guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogeneous RNases. The lysate is then mixed with ethanol and loaded on a purification column. The chaotropic salt and ethanol cause RNA to bind to the silica membrane while the lysate is spun through the column (2). Subsequently, impurities are effectively removed from the membrane by washing the column with wash buffers. Pure RNA is then eluted under low ionic strength conditions with nuclease-free water.

Specifications of RNASure Mini Kit

The Nucleopore™ RNA Purification Kit is a simple and efficient system for purification of total RNA from mammalian cultured cells, tissue, human blood cells, bacteria, yeast and insects. The kit utilizes a silica-based membrane technology in the form of a convenient spin column, eliminating the need for tedious cesium chloride gradients, alcohol precipitation or toxic phenol-chloroform extractions.

RNA molecules longer than 200 nucleotides can be isolated with the RNASure Mini Kit in 15 minutes after the lysis step. The high-quality purified RNA can be used in a wide range of downstream applications including RT-PCR, RT-qPCR, Northern blotting and other RNA-based analyses. See Table 1 for typical total RNA yields from various sources.

Starting Material and Typical Yield

Source	Quantity	Yield, μg
Mouse heart	20 mg	10-15
Mouse muscle	30 mg	8-10
Mouse lung	30 mg	25-30
Mouse kidney	30 mg	25-30
Mouse liver	30 mg	60-65
Mouse spleen	5 mg	10-15
<i>Bacillus pumilis</i> cells	1×10^9 cells	15-20
<i>Escherichia coli</i> cells	1×10^9 cells	25-30
HeLa cells	5×10^6 cells	35-40
Jurkat cells	5×10^6 cells	40-50
Cos7 cells	1×10^6 cells	20-25
<i>Saccharomyces cerevisiae</i> cells	4×10^8 cells	150-160

Handling Preparation and Storage of Samples

Proteinase K is stable at room temperature as long as not opened. After being opened it should be stored at -20°C . Other components of the kit should be stored at room temperature ($15-25^{\circ}\text{C}$). **Note. Close the bag with Nucleopore RNA Purification Columns tightly after each use**

Preparation and Storage of Reagents

NP-84105

Add the 10ml ethanol to 40ml of **Wash Buffer WBA2** (concentrated) and 39ml of ethanol to 23ml of **Wash Buffer WBA3** (concentrated) prior to first use.

NP-84107

Add the 50ml ethanol to 200ml of **Wash Buffer WBA2** (concentrated) and 170ml of ethanol to 100ml of **Wash Buffer WBA3** (concentrated) prior to first use.

After the ethanol has been added, mark the check box on the bottle's cover to indicate the completed step.

Proteinase K is supplied ready to use

- Before each RNA purification add 20 μL of 14.3 M β -mercaptoethanol or 20 μL of 2M DTT to each 1 mL volume of **Lysis Buffer LBA1** used.

Note: Check **Lysis Buffer LBA1** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.

Avoiding ribo nuclease 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 RNA preparation, especially during the column 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 (pipettes, centrifuges) and work surfaces.
- Keep all kit components tightly sealed when not in use. After usage close bottles immediately.

Starting material handling and storage

- When purifying RNA from fresh samples, place the samples on ice 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.
- Animal and bacterial cells can be pelleted and stored at -70°C until required. However, for RNA purification from yeast cells using enzymatic lysis, only freshly harvested samples can be used.
- Blood sample collection and RNA purification from blood cells should be carried out within the same day. Samples should be stored at 4°C until use. Do not freeze blood samples.

Reagents, Consumables and Equipments not Supplied with the Kit

- Pipettes and sterile, RNase-free pipette tips
- Vortex
- Microcentrifuge
- Microcentrifuge tubes
- Disposable gloves

- Equipment for sample disruption and homogenization (depending on the method chosen): EZLyzer, Mortar and pestle Rotor-stator homogenizer or Blunt needle and syringe etc.
- 14.3 M β -mercaptoethanol or 2 M DTT
- Ethanol (96-100%)

Other Buffers

For mammalian cultured cells lysate preparation:

PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, pH 7.4).

For gram-positive and gram-negative bacteria lysate preparation:

TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA) containing lysozyme (0.4mg/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 50 units of lyticase or zymolyase 20T just prior to use).

For Proteinase K dilution:

TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

SAFETY INSTRUCTIONS

Wear gloves when handling the **Lysis Buffer LBA1 and Wash Buffer WBA2** as these solutions contain irritants and are harmful if contacted with skin, inhaled or swallowed.

Lysis Solution

Contain Guanidinium thiocyanate: R&S Phrases: R22, 36/38, S23, 26, 36/37, 60

Proteinase K

Contain Proteinase, Tritirachium album serine: R&S Phrases: R42, S23, S36, S45, S60

R22: Harmful if swallowed eyes and skin.,R36/38: R42: May cause sensitization by inhalation, Irritating to eyes and skin S23: Do not breathe gas/fumes/vapour/spray, S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36/37: Wear suitable protective clothing and gloves, S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible), S60: This material and its container must be disposed of as hazardous waste.

RNA PURIFICATION PROTOCOLS

Protocols for RNA purification from mammalian cultured cells, tissues, insects, human blood, bacteria and yeast are described on p. 6-13.

Protocol for Total RNA Purification from Mammalian Tissue and Insect

Things to Do Before starting:

- Add required amount of Lysis Buffer LBA1 with β -mercaptoethanol or DTT. Add 20 μ L of 14.3 M β -mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer LBA1 required.
- Prepare the required amount of Proteinase K solution: dilute 10 μ L of Proteinase K (included) to 590 μ L of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

Procedure

1. **Homogenize up to 30 mg of fresh or frozen tissue, take the insect and disrupt the material by either using a mortar and pestle, EZ Lyzer, Tissue Lyzer or hand held homogenizers. Add 300ul of Lysis Buffer LBA1 with β -mercaptoethanol or DTT. Pulse vortex.**

Transfer the tissue or insect powder to the Lysis Buffer LBA1 as quickly as possible. Leaving the powder without with the Lysis Buffer LBA1 can result in degraded RNA. The grinded tissue or insect should be directly used for RNA purification and should not be stored.

2. **Add 600 μ L of diluted Proteinase K (10 μ L of the included Proteinase K diluted in 590 μ L of TE buffer). Vortex to mix thoroughly and incubate at 15-25°C for 10 min. Centrifuge for 5 min (if lysate is prepared from <10 mg of starting material) or 10 min (if lysate is prepared from >10 mg of starting material) at $\geq 12000 \times g$. Transfer the supernatant into a new RNase-free microcentrifuge tube (not included).**
3. **Add 450 μ L of ethanol (96-100%) and mix by pipetting. Transfer up to 700 μ L of lysate to the RNASure Mini Column inserted in a collection tube. Centrifuge the column for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.**
4. **Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the RNASure Mini Column into a new 2 mL collection tube (included).**

Note. Close the bag with RNASure Mini Columns tightly after each use!

5. **Add 700 μ L of Wash Buffer 1 (supplemented with ethanol, see p. 3) to the Nucleopore**

RNA Purification Column and centrifuge for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.

- 6. Add 600 μ L of Wash Buffer 2 (supplemented with ethanol, see p. 3) to the Nucleopore RNA Purification Column and centrifuge for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.**
- 7. Add 250 μ L of Wash Buffer 2 to the RNASure Mini Column and centrifuge for 2 min at $\geq 12000 \times g$.**
Optional. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed.
- 8. Discard the collection tube containing the flow-through solution and transfer the RNASure Mini Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).**
- 9. Add 100 μ L of Water, nuclease-free (included) to the center of the RNASure Mini Column membrane. Centrifuge for 1 min at $\geq 12000 \times g$ to elute RNA.**

Note

More than 90% of RNA is eluted during the first elution step. For maximum RNA yield repeat the elution step with an additional 100 μ L of Water, nuclease-free. If more concentrated RNA is required or RNA is isolated from a small amount of starting material (e.g., < 5 mg) the volume of Water, nuclease-free can be reduced to 50 μ L for the first and second elution steps. Please be aware that smaller volumes of eluant may result in a smaller final quantity of eluted RNA. Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

Protocol for Total RNA purification from Mammalian Cultured Cells

Before starting:

- Supplement the required amount of Lysis Buffer LBA1 with β -mercaptoethanol or DTT. Add 20 μ L of 14.3 M β -mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer LBA1 required.

Procedure

1. a) Suspension cells

Pellet up to 1×10^7 cells in an appropriate centrifuge tube by centrifugation for 5 min at 250 x 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 5×10^6 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 an appropriate volume of PBS or by trypsinization. Transfer the cells into a microcentrifuge tube (not included) and pellet them by centrifugation for 5 min at 250 x g. Discard the supernatant.

Note. Pelleted cells can be directly used for RNA isolation or stored at -70°C until use.

2. Resuspend the cells collected in step 1a or 1b in 600 μ l of Lysis Buffer LBA1 supplemented with β -mercaptoethanol or DTT. Vortex for 10 s to mix thoroughly.

- If after the addition of Lysis Buffer the mixture becomes viscous and dense, homogenization of the sample is required. Homogenize the lysate for 30 s using a rotor-stator homogenizer or pass the lysate through a blunt 20-gauge needle fitted to an RNase-free syringe several times. Incomplete homogenization will result in a significant reduction of RNA yields.
- If any cell debris is observed in the sample, centrifuge the tubes for 5 min at 14000 x g and transfer the supernatant into a new RNase-free microcentrifuge tube (not included).

3. Add 360 μ L of ethanol (96-100%) and mix the sample by pipetting.

Transfer up to 700 μ L of lysate to the RNASure Mini Column inserted in a collection tube. Centrifuge the column for 1 min at ≥ 12000 x g. Discard the flow-through and place the purification column back into the collection tube.

4. **Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the RNASure Mini Column into a new 2 mL collection tube (included).**

Note. Close the bag with RNASure Mini Columns tightly after each use!

5. **Add 700 μ L of Wash Buffer WBA2 to the Nucleopore RNA Purification Column and centrifuge for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.**

6. Add 600 μ L of Wash Buffer WBA3 (ethanol added) to the Nucleopore RNA Purification Column and centrifuge for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.

7. Add 250 μ L of Wash Buffer 2 to the RNASure Mini Column and centrifuge for 2 min at $\geq 12000 \times g$.

Optional. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min at maximum speed. Discard the collection tube containing the flow-through solution and transfer the RNASure Mini Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).

8. Add 100 μ L of Water, nuclease-free (included) to the center of the RNASure Mini Column membrane. Centrifuge for 1 min at $\geq 12000 \times g$ to elute RNA.

More than 90% of RNA is eluted during the first elution step. For maximum RNA yield, repeat the elution step with an additional 100 μ L of Water, nuclease-free. If more concentrated RNA is required or RNA is isolated from a small amount of starting material (e.g., $\leq 1 \times 10^6$ cells), the volume of the Water, nuclease-free can be reduced to 50 μ L for the first and second elution steps. Please be aware that smaller volumes of eluant may result in a smaller final quantity of eluted RNA. Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

Total RNA Purification from Human Blood Cells

Before starting:

- Supplement the required amount of Lysis Buffer LBA1 with β -mercaptoethanol or DTT. Add 20 μ L of 14.3 M β -mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer LBA1 required.
- Blood sample collection and RNA purification from blood cells should be carried out within the same day. Samples should be stored at 4°C until use. Do not freeze blood samples.

Procedure

1. **Collect blood cells by centrifugation of 0.5 mL of whole blood at 400 x g for 5 min at 4°C. Blood cells will generate a pellet of approximately 60-70% of the total sample volume. Remove the clear supernatant (plasma) from the pellet with a pipette. Resuspend the pellet in 600 μ L of Lysis Buffer LBA1 supplemented with β -mercaptoethanol or DTT. Vortex or pipet to mix thoroughly.**
2. **Add 450 μ L of ethanol (96-100%) and mix by pipetting.**
3. **Transfer up to 700 μ L of lysate to the RNASure Mini Column inserted in a collection tube. Centrifuge the column for 1 min at ≥ 12000 x g. Discard the flow-through and place the purification column back into the collection tube.**
4. **Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the RNASure Mini Column into a new 2 mL collection tube (included).**
Note. Close the bag with RNASure Mini Columns tightly after each use!
5. **Add 700 μ L of Wash Buffer 1 (supplemented with ethanol, see p. 3) to the Nucleopore**
6. **RNA Purification Column and centrifuge for 1 min at ≥ 12000 x g. Discard the flow-through and place the purification column back into the collection tube.**
7. **Add 600 μ L of Wash Buffer 2 (supplemented with ethanol, see p. 3) to the Nucleopore**

- 8. RNA Purification Column and centrifuge for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.**
- 9. Add 250 μL of Wash Buffer 2 to the RNASure Mini Column and centrifuge for 2 min at $\geq 12000 \times g$.**

Optional. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the RNASure Mini Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).
- 10. Add 50 μL of Water, nuclease-free (included) to the center of the Nucleopore RNA Purification Column membrane. Centrifuge for 1 min at $\geq 12000 \times g$ to elute RNA. Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.**

Bacteria Total RNA Purification Protocol

Important Note

For RNA isolation bacteria cells should be harvested during the exponential phase of growth (OD₆₀₀=0.5-1). **Do not use an overnight culture for RNA isolation.**

Before starting:

- 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.
- Supplement the required amount of Lysis Buffer LBA1 with β-mercaptoethanol or DTT. Add 20 μL of 14.3 M β-mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer LBA1 required.

Procedure

1. Transfer 1.5 mL of Gram-negative or Gram-positive bacterial culture (up to 1x10⁹ cells) to a 1.5 mL microcentrifuge tube. Collect cells by centrifugation for 2 min at ≥12000 x 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 (0.4mg/mL final concentration). Invert the tube several times to mix.
3. Incubate the resuspended cells for 5 min at 15-25°C.
4. Add 300 μL of Lysis Buffer LBA1 supplemented with β-mercaptoethanol or DTT. Mix thoroughly by vortexing for about 15 s until a homogeneous mixture is obtained.
5. Add 180 μL of ethanol (96-100%) and mix by pipetting. Transfer up to 700 μL of lysate to the RNASure Mini Column inserted in a collection tube. Centrifuge the column for 1 min at ≥12000 x g. Discard the flow-through and place the purification column back into the collection tube.
6. Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the RNASure Mini Column into a new 2 mL collection tube (included).
Note. Close the bag with RNASure Mini Columns tightly after each use!
7. Add 700 μL of Wash Buffer 1 (supplemented with ethanol, see p. 3) to the Nucleopore RNA Purification Column and centrifuge for 1 min at ≥12000 x g. Discard the flow-through and place the purification column back into the collection tube.
8. Add 600 μL of Wash Buffer 2 (supplemented with ethanol, see p. 3) to the Nucleopore RNA Purification Column and centrifuge for 1 min at ≥12000 x g. Discard the flow-through and place the purification column back into the collection tube.

9. Add 250 μ L of Wash Buffer 2 to the RNASure Mini Column and centrifuge for 2 min at $\geq 12000 \times g$.

Optional. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the RNASure Mini Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).

10. Add 100 μ L of Water, nuclease-free (included) to the center of the Nucleopore RNA Purification Column membrane. Centrifuge for 1 min at $\geq 12000 \times g$ to elute RNA.

More than 90% of RNA is eluted during the first elution step. For maximum RNA yield repeat the elution step with an additional 100 μ L of Water, nuclease-free. If more concentrated RNA is required or RNA is isolated from a small amount of starting material (e.g., $\leq 1 \times 10^6$ cells) the volume of the Water, nuclease-free can be reduced to 50 μ L for the first and second elution. Please be aware that smaller volumes of eluant may result in smaller final quantity of eluted RNA. Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

Protocol for Total RNA Purification Yeast

For RNA isolation yeast cells should be harvested at the exponential phase of growth (OD₆₀₀=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 50 units of lyticase or zymolyase 20T.
- Supplement the required amount of Lysis Buffer LBA1 with β-mercaptoethanol or DTT. Add 20 μL of 14.3 M β-mercaptoethanol or 2 M DTT to each 1 mL volume of Lysis Buffer LBA1 required.
- Prepare the required amount of Proteinase K solution: dilute 10 μL of Proteinase K (included) to 590 μL of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

Step Procedure

1. Transfer the yeast cell culture (up to 4×10^8 yeast cells) to a 1.5 mL microcentrifuge tube (not provided). Collect the cells by centrifugation for 2 min at $\geq 12000 \times g$. Discard the supernatant.
2. Resuspend the cell pellet in 100 μL of Yeast lysis buffer LBA1.
3. Incubate at 30°C for 30 min.
4. Add 300 μL of Lysis Buffer LBA1 supplemented with β-mercaptoethanol or DTT. Mix thoroughly by vortexing or pipetting.
5. Add 600 μL of diluted Proteinase K (10 μL of the included Proteinase K diluted in 590 μL of TE buffer). Vortex to mix thoroughly and incubate at 15-25°C for 10 min.
6. Centrifuge for 10 min at $\geq 12000 \times g$. Transfer the supernatant into a new RNase-free microcentrifuge tube (not included).
7. Add 450 μL of ethanol (96-100%) and mix by pipetting.
8. Transfer up to 700 μL of lysate to the RNASure Mini Column inserted in a collection tube. Centrifuge the column for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.
9. Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the RNASure Mini Column into a new 2 mL collection tube (included).
Note. Close the bag with RNASure Mini Columns tightly after each use!
10. Add 700 μL of Wash Buffer 1 (supplemented with ethanol, see p. 3) to the Nucleopore RNA Purification Column and centrifuge for 1 min at $\geq 12000 \times g$.

Discard the flow-through and place the purification column back into the collection tube.

11. Add 600 μL of Wash Buffer 2 (supplemented with ethanol, *see p. 3*) to the Nucleopore RNA Purification Column and centrifuge for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.
12. Add 250 μL of Wash Buffer 2 to the RNASure Mini Column and centrifuge for 2 min at $\geq 12000 \times g$.

Optional. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the RNASure Mini Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included). Add 100 μL of Water, nuclease-free (included) to the center of the RNASure Mini Column membrane. Centrifuge for 1 min at $\geq 12000 \times g$ to elute RNA.

Note

- More than 90% of RNA is eluted during the first elution step. For maximum RNA yield repeat the elution step with an additional 100 μL of Water, nuclease-free.
- If more concentrated RNA is required or RNA is isolated from a small amount of starting material the volume of Water, nuclease-free can be reduced to 50 μL for the first and second elution steps. Please be aware that smaller volumes of eluant may result in a smaller final quantity of eluted RNA.

13. Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

Genomic DNA Removal from RNA Preparations

Using DNase I, stand-alone product

Procedure

Add to an RNase-free tube:

RNA	1µg
10X reaction buffer with MgCl ₂	1µl
DNase I, RNase-free	1µl
DEPC-treated Water	to 10 µl

Incubate at 37°C for 30 min.

Note. RNase Inhibitor can be included in the reaction mixture to prevent RNA degradation.

Add 1 µL 50 mM EDTA and incubate at 65°C for 10 min to inactivate the DNase I. Addition of EDTA is required as RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent. Alternatively, repurify RNA using the RNASure Mini Kit (see p. 15 for RNA Cleanup Protocol) or perform a phenol/chloroform extraction.

Use the prepared RNA for downstream applications.

Note

- Do not use more than 1 u of DNase I per 1 µg of RNA.
- Volumes of the reaction mixture and 50 mM EDTA solution can be scaled up for larger amounts of RNA. The recommended final concentration of RNA is 0.1 µg/µL.

RNA Cleanup Protocol

Nucleopore™ RNA Purification Kit can be used to clean up to 100 µg RNA cleanup after DNase I digestion or other enzymatic reactions.

Procedure

1. Adjust the volume of the reaction mixture to 100 µL with Water, nuclease-free (included). Add 300 µL of Lysis Buffer LBA1 without β-mercaptoethanol or DTT. Mix thoroughly by vortexing or pipetting.
2. Add 180 µL of ethanol (96-100%) and mix by pipetting.

3. Transfer the mixture to the RNASure Mini Column inserted in a collection tube. Centrifuge the column for 1 min at $\geq 12000 \times g$. Discard the collection tube containing the flow-through solution. Place the RNASure Mini Column into a new 2 mL collection tube (included).

Note. Close the bag with RNASure Mini Columns tightly after each use!

4. Add 700 µL of Wash Buffer 1 (supplemented with ethanol, see p. 3) to the Nucleopore RNA Purification Column and centrifuge for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.

5. Add 600 µL of Wash Buffer 2 (supplemented with ethanol, see p. 3) to the Nucleopore RNA Purification Column and centrifuge for 1 min at $\geq 12000 \times g$. Discard the flow-through and place the purification column back into the collection tube.

6. Add 250 µL of Wash Buffer 2 to the RNASure Mini Column and centrifuge for 2 min at $\geq 12000 \times g$.

Optional. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min at maximum speed. Discard the collection tube containing the flow-through solution and transfer the RNASure Mini Column to a sterile 1.5 mL RNase-free microcentrifuge tube (included).

7. Add 50 µL of Water, nuclease-free (included) to the center of the Nucleopore RNA Purification Column membrane. Centrifuge for 1 min at $\geq 12000 \times g$ to elute RNA.
Note. For maximum RNA yield repeat the elution step with an additional 50 µL of Water, nuclease-free. Discard the purification column.
8. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

TROUBLESHOOTING

Problem	Possible cause and solution
<p>Low RNA yield</p>	<p>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.</p> <p>Starting material was not completely disrupted. Reduce the amount of starting material. Increase disruption time.</p> <p>Ethanol was not added to the lysate. Make sure that ethanol was added to the lysate before applying the sample to the purification column.</p> <p>Ethanol was not added to Wash Buffers 1 and 2. Make sure that ethanol was added to Wash Buffers WBA2 and WBA3 prior to the first use.</p>
<p>Degraded RNA Degraded RNA</p>	<p>Inappropriate handling of starting material. When purifying RNA from fresh samples, place samples on ice immediately after harvesting. Proceed to lysis and homogenization as quickly as possible. Ensure that frozen 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.</p> <p>RNase contamination. To avoid RNase contamination, wear gloves during all procedure and change gloves frequently. Use sterile, disposable RNase-free pipette tips. Remove RNase contamination from non-disposable items and work surfaces.</p>
<p>DNA contamination</p>	<p>Digest RNA preparation with DNase I. Inactivate DNase I by heat treatment (in the presence of a chelating agent), phenol/chloroform extraction or re-purify RNA following RNA Cleanup Protocol</p>
<p>Column clogging</p>	<p>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.</p> <p>Starting material was not completely disrupted. Reduce the amount of starting material and increase disruption time.</p> <p>Tissue debris was not removed before applying lysate on a column (Mammalian Tissue and Insect Total RNA Purification Protocol). Centrifuge the disrupted and homogenized cell suspension to remove tissue debris prior to applying the lysate to the column.</p>
<p>Enzymatic reactions not running well</p>	<p>Ethanol was carried over into the eluted RNA. If residual solution is seen in the purification column after washing with Wash Buffer WBA3, empty the collection tube and re-spin the column for an additional 1 min at maximum speed ($\geq 12000 \times g$).</p> <p>Salt was carried over into the eluted RNA. Use the Wash Buffers the correct order. Always wash the purification column with Wash Buffer WBA2 first and then proceed to wash with Wash Buffer WBA3.</p>



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