SciPhi™

PRODUCT INFORMATION

RNA Purification Kit

#NXG992

Read Storage and Reagent preparation information before first use.

For Research Use Only. Not recommended for diagnostic use.

Kit Components:

Component	Quantity
RNA Purification Columns with Collection Tubes	50
Collection Tubes (2 ml)	50
Microcentrifuge Tubes (1.5 ml)	50
Lysis Buffer	40 ml
Wash Buffer I (Conc.)	40 ml
Wash Buffer II (Conc.)	23 ml
Nuclease free Water	30 ml
Ethanol	75 ml
Proteinase K	0.6 ml

Storage Instructions:

As long as proteinase K solution is not opened, it is stable at room temperature. Once it has been opened, it needs to be kept at -20 °C. Other components of the kit can be kept at room temperature (15 to 25°C).
Please remember to properly close the bag containing SciPhi™ RNA Purification Columns after each use.

Product Description:

SciPhi™ RNA Purification Kit is suitable for fast and efficient total RNA purification from whole blood, mammalian cell culture, tissue samples, bacteria, yeast and insects. This kit is based on spin column silica membrane technology. Hence, no resins, no cesium chloride gradient, no phenol-chloroform extractions, and no alcohol precipitations are needed. It can purify RNA in 15 minutes after lysis with a yield of 10-160 µg (Table 1). Purified RNA from the kit is ideal for RT-PCR, qPCR, Northern blotting and other RNA based applications.

Table 1: Typical RNA yields from various sample sources

Source	Quantity	Yield, µg
Mouse Heart	20 mg	10-15
Mouse Muscle	30 mg	8-10
Mouse Lung	30 cm	25-30
Mouse Liver	30 mg	60-65
Mouse Spleen	5 mg	10-15
Mouse Kidney	30 mg	25-30
Bacillus pumilis cells	1 X 10^9 cells	15-20
Escherichia coli cells	1 X 10^9 cells	25-30
Cos7 cells	1 X 10^6 cells	20-25
HeLa cells	5 X 10^6 cells	35-40
Jurkat cells	5 X 10^6 cells	40-45
Saccharomyces cerevisiae cells	4 X 10^8 cells	150-160

Principle:

Digestion/Lysis: Depending on the initial material, the samples are subjected to Lysis Buffer. The lysis buffer contains guanidine thiocyanate which is capable to protect RNA from RNases.

Binding and Purification: The lysate (resulting mixture) is then mixed with ethanol and loaded onto a purification column. In this step, the RNA molecules present in the lysate attach to the silica membrane within the column, while impurities are effectively washed away using prepared wash buffers.

Elution: Total RNA is then can be eluted from the purification column under low ionic strength conditions using an Elution Buffer. This allows the RNA to be collected in a separate solution for further analysis or use.

Important Information

1) Buffer Preparations

a) Prepare **Wash Buffer I** and **Wash Buffer II** according to Table 2 before first use:

Table 2: Instruction to prepare wash buffers

	Wash Buffer I	Wash Buffer II
Concentrated Wash Buffer	40	23
Ethanol (96-100%)	10	39
Total Volume	**50**	**62**

Once the ethanol has been added, tick the box on the bottle's cap to indicate that the step has been completed.

b) Prepare **Lysis Buffer** before each experiment by adding 20 µl of **14.3 M β-Mercaptoethanol** or 20 µl of **DTT** to 1 ml of Lysis Buffer. Also, before each usage, check the Lysis Buffer for salt precipitation. If any precipitation observed, warm the buffer to 37°C to dissolve any precipitate, then let it cool to 25°C before using.

c) Buffer for mammalian cell lysate preparation:

i) Phosphate Buffer Saline (PBS): 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4.

d) Buffer for gram-positive and gram-negative bacteria lysate preparation:

i) Tris-EDTA (TE) Buffer with lysozyme: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. Add lysozyme with a final concentration of 0.4mg/ml.

e) Buffer for yeast lysate preparation:

i) **Yeast Lysis Buffer:** 1 M sorbitol, 0.1 M EDTA, pH 7.4. Add 50 units of Zymolyase 20T or Lysticase and 0.1% β-Mercaptoethanol, before use.

f) Buffer for Proteinase K Dilution:

i) **TE Buffer:** 10 mM Tris-HCl, pH 8.0, 1 mM EDTA

2) Buffer Handling

a) Handle **Wash Buffer I** and **Lysis buffer** after wearing the gloves as these buffers contains irritants and may cause harmful effects on skin if not handled properly.

3) Instructions to avoid RNase contamination:

a) Skin is a very common source of RNase. Hence it is recommended to wear gloves during the whole procedure of RNA isolation. Changing of gloves frequently is also a good way to avoid RNase contamination.

b) Always use sterile, disposable pipette tips that are free from RNase contamination.

c) Make use of appropriate reagents to eliminate RNase contamination from non-disposable items such as pipettes and centrifuges, as well as work surfaces.

d) Ensure that all components of the RNA kit are tightly sealed when not in use.

e) Immediately close all the bottles after each use.

4) Instructions for Handling and Storage od Starting Material:

a) Place the freshly harvested samples on ice immediately after collection. Proceed with lysis and homogenization as soon as possible.

b) Minimize the time between death and sample collection to ensure high-quality RNA isolation from sacrificed animals or cadavers.

c) Freeze the samples in liquid nitrogen and store them at -70°C, if RNA purification cannot be performed immediately after tissue collection. Do not thaw the frozen tissue samples during handling or weighing.

d) Pellet down the animal and bacterial cells and store at -70°C until needed.

e) When isolating RNA from yeast cells using enzymatic lysis, use only freshly harvested samples.

f) Carry out the RNA purification from blood cells on the same day of collection. Store blood samples at 4°C until use and avoid freezing blood samples.

Additional Instruments and Consumables Required

- Pipettes
- Sterile pipette tips
- Vortex Mixture
- Microcentrifuge
- Water Bath or Heat Block
- 2 M DTT / 14.3 M β-mercaptoethanol
- Lysozyme
- Equipment for sample lysis and homogenization:
  - Mortar and Pestle
  - Rotor-stator homogenizer
  - Blunt needle and syringe
- Disposable Gloves
- 1.5 ml Microcentrifuge tubes

PROTOCOLS FOR RNA ISOLATION

RNA isolation protocol from cultured mammalian cells, tissues, insects, mammalian blood, bacteria and yeast are described on page no. 5-12

RNA Isolation from Mammalian Tissue and Insect:

1. Prepare following reagents before starting the RNA isolation procedure:
   i. Add β-mercaptoethanol to Lysis buffer (supplied with kit) as per the instructions given on **Page No. 4, Section 1b**.
   ii. Prepare proteinase K solution by diluting 10 µl of Proteinase K with 590 µl of TE buffer for required number of samples. Prepare TE buffer for Proteinase K dilution as per the instructions given on **Page No. 4, Section 1f**.
2. Disrupt up to 30 mg of fresh or frozen mammalian tissue (use up to 10 mg of spleen tissue) by using any of the following method:
   i. By using a mortar and pestle: Subject the tissue sample or insect to liquid nitrogen and grind the sample using a mortar and pestle. Transfer the grounded sample to a fresh 1.5 ml microcentrifuge tube containing 300 µl of Lysis Buffer supplemented with β-mercaptoethanol or DTT. Mix the suspension by vortexing for 10 seconds. It should be followed by homogenization of the lysate using a rotor-stator homogenizer. Alternatively, repeatedly pass the prepared lysate into an RNase-free syringe using a blunt 20-gauge needle.

   Note:
   - Be as quick as possible while transferring the grounded sample to microcentrifuge tube as delay can cause the degradation of RNA.
   - Do not store the grounded sample. It should be immediately processed for RNA isolation.
   - Thoroughly mix all the grounded sample with lysis buffer. The sample should not allow to dry on the walls of the tube as it may lead to degradation of RNA.

   ii. By using a rotor-stator homogenizer: Add up to 30 mg of tissue (or up to 10 mg of spleen tissue) or insect to a properly sized vessel for homogenization. The vessel should contain 300 µL of Lysis Buffer, which is supplemented with β-mercaptoethanol or DTT. Proceed to homogenize the material immediately using a conventional rotor-stator homogenizer for 20-40 seconds or until the suspension achieves a uniform consistency.
3. Add 600 µl of already prepared Proteinase K Solution from step 1(ii). Mix thoroughly by vortexing and incubate for 10 minutes at room temperature.
4. Centrifuge at ≥12000 × g for 5 minutes (If starting material was <10 mg) or for 10 minutes (if starting material was >10 mg).
5. Discard the pellet and transfer the supernatant to a new RNase free microcentrifuge tube containing 450 µl of ethanol (96-100%). Mix carefully by pipetting.
6. Dispense 700 µl of the prepared lysate into the SciPhi™ RNA Purification Columns and centrifuge at ≥12000 x g for 1 minute. Repeat the step until whole lysate has been transferred to the column. Do not forget to discard flow though before transferring the

lysate to the column. Once all the lysate has been transferred to the column and centrifuge, discard the collection tube and place the column to a new collection tube.

Note: Remember to tightly seal the bag of SciPhi™ RNA Purification Columns tightly after each use!

7. Add 700 µl of Wash Buffer I (with ethanol added) to the column and centrifuge at ≥12000 x g for 1 minute. Discard the flow-through and place the purification column back into the collection tube.
8. Add 600 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at ≥12000 × g for 1 minutes.
9. Repeat the step with 250 µl of Wash Buffer II and centrifuge at ≥12000 × g for 1 minutes.

   Optional: If residual solution remains in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
10. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile RNase-free 1.5 ml microcentrifuge tube.
11. Elute RNA by adding 100 µl of nuclease-free water to the centre of the SciPhi™ RNA Purification Column membrane followed by centrifugation at ≥12000 × g for 1 minute.

    Note

    • For maximum RNA yield, repeat the elution step with additional 100 µl of nuclease-free water.

    • If more concentrated RNA is needed or RNA is isolated from a small amount of starting material (e.g., <5 mg of tissue) the volume of the nuclease-free water added to the column can be reduced to 50 µl for both the elution steps. However, be aware that smaller volumes of nuclease free water will yield a smaller final quantity of eluted RNA.
12. Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20 °C or -70 °C until use.

RNA Isolation from Cultured Mammalian Cells

1. Prepare Lysis buffer by adding β-mercaptoethanol to Lysis buffer (supplied with kit) as per the instructions given on Page No. 4, Section 1b.
2. **For Suspension Cells**: Pellet down up to 1 × 10^7 cells in a centrifuge tube by centrifugation at 250 × g for 5 min. Discard the supernatant and rinse pelleted cells once with PBS to remove residual medium and repeat the centrifugation step. Discard the supernatant.

   For Adherent Cells: Remove the growth medium from a culture plate containing up to 5 × 10^6 cells. Rinse cells once with PBS to remove residual medium. Discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer the cells to a microcentrifuge tube and pellet them by centrifugation at 250 × g for 5 minutes. Discard supernatant.

   Note: User can use pelleted cell directly for RNA isolation or can store at -70°C for later use.
3. Resuspend the pelleted cells in 600 µl of Lysis Buffer containing β-mercaptoethanol. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.

   Note:

   - Homogenization of the sample is necessary if the mixture becomes dense and viscous after the addition of Lysis Buffer. Use a rotor-stator homogenizer and homogenize the lysate for 30 seconds. Alternatively, or repeatedly run it through a blunt 20-gauge needle attached to an RNase-free syringe. RNA yield may be got reduced due to incomplete homogenization.
   - If any visible cell debris is found in sample, centrifuge the sample at 14000 X g for 5 minutes and transfer the supernatant into a fresh RNase-free microcentrifuge tube.
4. Add 360 µl of 96-100% ethanol and mix by pipetting or vortexing.
5. Dispense 700 µl of the prepared lysate into the SciPhi™ RNA Purification Columns and centrifuge at ≥12000 x g for 1 minute. Repeat the step until whole lysate has been transferred to the column. Do not forget to discard flow though before transferring the lysate to the column. Once all the lysate has been transferred to the column and centrifuge, discard the collection tube and place the column to a new collection tube.

   Note: Remember to tightly seal the bag of SciPhi™ RNA Purification Columns tightly after each use!
6. Add 700 µl of Wash Buffer I (with ethanol added) to the column and centrifuge at ≥12000 x g for 1 minute. Discard the flow-through and place the purification column back into the collection tube.
7. Add 600 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at ≥12000 × g for 1 minutes.
8. Repeat the step with 250 µl of Wash Buffer II and centrifuge at ≥12000 × g for 1 minutes.

   Optional: If residual solution remains in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
9. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile RNase-free 1.5 ml microcentrifuge tube.
10. Elute RNA by adding 100 µl of nuclease-free water to the centre of the SciPhi™ RNA Purification Column membrane followed by centrifugation at ≥12000 × g for 1 minute.

    Note:

- For maximum RNA yield, repeat the elution step with additional 100 µl of nuclease-free water.
- If more concentrated RNA is needed or RNA is isolated from a small amount of starting material (e.g., <5 mg of tissue) the volume of the nuclease-free water added to the column can be reduced to 50 µl for both the elution steps. However, be aware that smaller volumes of nuclease free water will yield a smaller final quantity of eluted RNA.

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

RNA Isolation from Mammalian Blood

Note: Carry out the RNA purification from blood cells on the same day of collection. Store blood samples at 4°C until use and avoid freezing blood samples.

1. Pellet down the blood cells by centrifugation of 0.5 ml of whole blood for 5 minutes at 400 x g at 4 °C. Centrifugation will result in generation of a pellet of 60-70% of total blood sample volume. Carefully remove and discard the supernatant (plasma) with a pipette.
2. Add 600 µl of Lysis Buffer (containing β-mercaptoethanol) to the pellet and mix thoroughly by pipetting or vortexing.
3. Add 450 µl of ethanol (96-100%) and mix by pipetting or vortexing.
4. Dispense 700 µl of the prepared lysate into the SciPhi™ RNA Purification Columns and centrifuge at ≥12000 x g for 1 minute. Repeat the step until whole lysate has been transferred to the column. Do not forget to discard flow though before transferring the lysate to the column. Once all the lysate has been transferred to the column and centrifuge, discard the collection tube and place the column to a new collection tube.

   Note: Remember to tightly seal the bag of SciPhi™ RNA Purification Columns tightly after each use!
5. Add 700 µl of Wash Buffer I (with ethanol added) to the column and centrifuge at ≥12000 x g for 1 minute. Discard the flow-through and place the purification column back into the collection tube.
6. Add 600 µl of Wash Buffer II (with ethanol added) to the column and centrifuge at ≥12000 × g for 1 minutes.
7. Repeat the step with 250 µl of Wash Buffer II and centrifuge at ≥12000 × g for 1 minutes.

   Optional: If residual solution remains in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
8. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile RNase-free 1.5 ml microcentrifuge tube.
9. Elute RNA by adding 50 µl of nuclease-free water to the centre of the SciPhi™ RNA Purification Column membrane followed by centrifugation at ≥12000 × g for 1 minute.

   Note:

   - For maximum RNA yield, repeat the elution step with additional 100 µl of nuclease-free water.
   - If more concentrated RNA is needed or RNA is isolated from a small amount of starting material (e.g., <5 mg of tissue) the volume of the nuclease-free water added to the column can be reduced to 50 µl for both the elution steps. However, be aware that smaller volumes of nuclease free water will yield a smaller final quantity of eluted RNA.
10. Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20 °C or -70 °C until use.

Total RNA Isolation from Bacteria

Note: Harvest the bacterial cells only from exponential phase (OD_{600}=0.5-1) for RNA isolation. Avoid using overnight cultures for RNA isolation.

1. Pellet down 1.5 ml bacterial culture containing up to 1 × 10^9 bacterial cells by centrifugation at ≥12000 × g for 2 minutes and discard the supernatant. Make sure to completely remove the supernatant.
2. Resuspend the pellet in 100 µl of freshly prepared TE buffer consisting lysozyme with a final concentration of 0.4mg/ml. Mix by inverting the tube numerous times.
3. Incubate the suspension at 15-25°C for 5 minutes.
4. Add 300 µl of Lysis Buffer (containing β-mercaptoethanol) to the suspension and mix thoroughly by pipetting or vortexing.
5. Add 180 µl of ethanol (96-100%) and mix by pipetting or vortexing.
6. Dispense 700 µl of the prepared lysate into the SciPhi™ RNA Purification Columns and centrifuge at ≥12000 x g for 1 minute. Repeat the step until whole lysate has been transferred to the column. Do not forget to discard flow though before transferring the lysate to the column. Once all the lysate has been transferred to the column and centrifuge, discard the collection tube and place the column to a new collection tube.

   Note: Remember to tightly seal the bag of SciPhi™ RNA Purification Columns tightly after each use!
7. Add 700 µl of Wash Buffer I (with ethanol added) to the column and centrifuge at ≥12000 x g for 1 minute. Discard the flow-through and place the purification column back into the collection tube.
8. Add 600 µl of Wash Buffer II (with ethanol added) to the column and centrifuge at ≥12000 × g for 1 minutes.
9. Repeat the step with 250 µl of Wash Buffer II and centrifuge at ≥12000 × g for 1 minutes.

   Optional: If residual solution remains in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
10. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile RNase-free 1.5 ml microcentrifuge tube.
11. Elute RNA by adding 100 µl of nuclease-free water to the centre of the SciPhi™ RNA Purification Column membrane followed by centrifugation at ≥12000 × g for 1 minute.

    Note:

    - For maximum RNA yield, repeat the elution step with additional 100 µl of nuclease-free water.
    - If more concentrated RNA is needed or RNA is isolated from a small amount of starting material (e.g., <5 mg of tissue) the volume of the nuclease-free water added to the column can be reduced to 50 µl for both the elution steps. However, be aware that smaller volumes of nuclease free water will yield a smaller final quantity of eluted RNA.
12. Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20 °C or -70 °C until use.

DNA Isolation from Yeast

Note: Harvest the yeast cells only from exponential phase (OD_{600}=0.5-1) for RNA isolation. Avoid using overnight cultures for RNA isolation.

1. Prepare following reagents before starting the RNA isolation procedure:
   - Prepare Yeast lysis buffer before starting: 1 M sorbitol, 0.1 M EDTA, pH 7.4. Just before using the yeasts lysis buffer, add 5 mg/mL zymolyase 20T,
   - Add β-mercaptoethanol to Lysis buffer (supplied with kit) as per the instructions given on Page No. 4, Section 1b.
   - Prepare proteinase K solution by diluting 10 µl of Proteinase K with 590 µl of TE buffer for required number of samples. Prepare TE buffer for Proteinase K dilution as per the instructions given on Page No. 4, Section 1f.
2. Pellet down up to 4×10^8 yeast cells in a 1.5 / 2 mL microcentrifuge tube by centrifugation at ≥12000 × g for 2 minutes and discard the supernatant
3. Resuspend the pellet in 100 µl of Yeast lysis buffer and incubate the microcentrifuge tube at 30°C for 30 minutes.
4. Add 300 µl of Lysis Buffer (containing β-mercaptoethanol) to the suspension and mix thoroughly by pipetting or vortexing.
5. Add 600 µl of Proteinase K (diluted as described above in Point 1), vortex and incubate for 10 minutes at 15-25°C.
6. Centrifuge the suspension at ≥12000 × g for 10 minutes and transfer the supernatant to a new sterile, RNase-free microcentrifuge tube.
7. Add 450 µl of 96-100% ethanol and mix gently by pipetting.
8. Dispense 700 µl of the prepared lysate into the SciPhi™ RNA Purification Columns and centrifuge at ≥12000 x g for 1 minute. Repeat the step until whole lysate has been transferred to the column. Do not forget to discard flow though before transferring the lysate to the column. Once all the lysate has been transferred to the column and centrifuge, discard the collection tube and place the column to a new collection tube.

   Note: Remember to tightly seal the bag of SciPhi™ RNA Purification Columns tightly after each use!
9. Add 700 µl of Wash Buffer I (with ethanol added) and centrifuge at ≥12000 x g for 1 minute. Discard the flow-through and place the purification column back into the collection tube
10. Add 600 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at ≥12000 × g for 1 minute.
11. Repeat the step with 250 µl of Wash Buffer II and centrifuge at ≥12000 × g for 1 minutes.

    Optional: If residual solution remains in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
12. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile RNase-free 1.5 ml microcentrifuge tube.
13. Elute RNA by adding 100 µl of nuclease-free water to the centre of the SciPhi™ RNA Purification Column membrane followed by centrifugation at ≥12000 × g for 1 minute.

    Note:

- For maximum RNA yield, repeat the elution step with additional 100 µl of nuclease-free water.
- If more concentrated RNA is needed or RNA is isolated from a small amount of starting material (e.g., <5 mg of tissue) the volume of the nuclease-free water added to the column can be reduced to 50 µl for both the elution steps. However, be aware that smaller volumes of nuclease free water will yield a smaller final quantity of eluted RNA.

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

ADDITIONAL PROTOCOLS

Protocol for DNase treatment*

1. Prepare the following DNase reaction mixture and add to an RNase free microcentrifuge tube:
    - RNA : 1-10 µg
    - Reaction Buffer : 1X
    - DNase I : 1-2 unit
    - DEPC treated water : up to 10 -100 µl
2. Incubate the reaction at 37°C for 10 - 30 minutes.
3. Add 50mM EDTA (to a final concentration of 5mM) to the reaction.
4. Incubate for 10 minutes at 65°C - 75°C to inactivate the DNase I.

***Note:** This protocol outlines the procedure for DNase treatment in a general context. The concentration of enzymes and other reagents may vary according to different manufacturers/brands. Hence, users are advised to use brand specific DNase treatment protocol for successful DNase I treatment.

Protocol for RNA Cleanup

Note: SciPhi™ RNA Purification Kit can cleanup the RNA upto 100 µg.

1. Bring the volume of the reaction mixture to 100 µL using included nuclease-free Water.
2. Add 300 µl of Lysis Buffer (**without β-mercaptoethanol**) to the suspension and mix thoroughly by pipetting or vortexing.
3. Add 180 µl of 96-100% ethanol, mix gently by pipetting and transfer the suspension to SciPhi™ RNA Purification Columns.
4. Centrifuge the column at ≥12000 x g for 1 minute. Repeat the step until whole lysate has been transferred to the column. Discard the collection tube and place the column to a new collection tube.

   Note: Remember to tightly seal the bag of SciPhi™ RNA Purification Columns tightly after each use!
5. Add 700 µl of Wash Buffer I (with ethanol added) and centrifuge at ≥12000 x g for 1 minute. Discard the flow-through and place the purification column back into the collection tube
6. Add 600 µl of Wash Buffer II (with ethanol added) to the Column and Centrifuge at ≥12000 × g for 1 minute.
7. Repeat the step with 250 µl of Wash Buffer II and centrifuge at ≥12000 × g for 2 minutes.

   Optional: If residual solution remains in the purification column, empty the collection tube and re-spin the column for 1 minute at maximum speed.
8. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile RNase-free 1.5 ml microcentrifuge tube.
9. Elute RNA by adding 50 µl of nuclease-free water to the centre of the SciPhi™ RNA Purification Column membrane followed by centrifugation at ≥12000 × g for 1 minute.
10. **Note:**
    - For maximum RNA yield, repeat the elution step with additional 50 µl of nuclease-free water.
11. Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20 °C or -70 °C until use.

TROUBLESHOOTING

Problem	Possible Cause	Solution
Low yield of purified RNA	Excess sample used during lysate preparation	Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocols
	Starting material was not completely digested	Extend the incubation time for cell disruption.
	Ethanol was not added to the lysate	Ensure the addition of ethanol to the lysate prior to applying the sample onto the purification column.
	Ethanol was not added to Wash Buffers.	Make sure that ethanol was added to Wash Buffer I and Wash Buffer II before use. Follow the instructions for Wash Buffer preparation on page no. 4
Purified RNA is degraded	Inappropriate sample handling	• Upon harvesting fresh samples for RNA purification, immediately transfer them onto ice. • Start the lysis and homogenization processes with minimal delay. • Make sure the frozen samples must be frozen in liquid nitrogen immediately after collection and subsequently store them at -70°C. • It is advised to prevent thawing of samples until the introduction of Lysis Buffer.
	RNase Contamination	• Wear gloves during the whole procedure of RNA isolation. Changing of gloves frequently is also a good way to avoid RNase contamination. • Use sterile, disposable RNase-free pipette tips. • Make use of appropriate reagents to remove RNase contamination from non-disposable items such as pipettes and centrifuges, as well as work surfaces.
DNA Contamination	Inappropriate DNase Treatment	Carry out DNase I treatment step as described on page 14.
Column becomes clogged during purification	Excess sample was used during lysate preparation.	Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocols.
	Sample was not completely lysed	Extend the incubation time for cell disruption.

	Sample debris was not removed before applying lysate on a column	Before applying the lysate to the column, centrifuge the disrupted and homogenized cell suspension in order to eliminate tissue debris.
Inhibition of downstream enzymatic reactions	Purified RNA contains residual ethanol.	If residual solution is seen in the purification column after washing the column with Wash Buffer II, empty the collection tube and re-spin the column for an additional 1 min. at maximum speed (≥12000 × g).
	Purified RNA contains residual salt	Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer I first and then proceed to washing with Wash Buffer II.

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