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GeneJET™ RNA Purification Kit

USER GUIDE

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For Research Use Only. Not for use in diagnostic procedures.

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Revision history: MAN0012664 B (English)

Revision	Date	Description
B	26 March 2025	• Updated to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. • The contents and storage table was updated to include additional instructions for storage of purification columns.
A	17 October 2015	Baseline for revision history.

The information in this guide is subject to change without notice.

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GeneJET RNA Purification Kit

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The GeneJET™ 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 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.

Technology overview

Samples are lysed and homogenized in Lysis Buffer, which contains guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogenous 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. After, 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.

Typical RNA yield

Table 1 Typical total RNA yields from various sources

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

Contents and storage

Table 2 Components of the GeneJET™ RNA Purification Kit

Item	Cat. No. K0731 (50 reactions)	Cat. No. K0732 (250 reactions)	Storage
Proteinase K	600 µL	5 × 600 µL	15–25°C [1]
Lysis Buffer	40 mL	200 mL	15–25°C
Wash Buffer I (concentrated)	40 mL	200 mL	
Wash Buffer II (concentrated)	23 mL	100 mL	
Water, nuclease-free	30 mL	125 mL	
GeneJET™ RNA Purification Columns (pre-assembled with Collection Tubes)	50	250	15–25°C For better long-term performance store at 2°C to 8°C.
Collection Tubes, 2 mL	50	250	15–25°C
Collection Tubes, 1.5 mL	50	250	

[1] Proteinase K is stable at 15–25°C if not opened. After opening, store Proteinase K at -20°C.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Equipment	
Adjustable micropipettors	**MLS**
Multi-channel micropipettors	**MLS**
Vortex mixer, or equivalent	**MLS**
Thermo heating-blocks or water bath (adjustable to 56°C)	**MLS**
Centrifuge capable of ≥20,000 × *g* for 1.5 mL microcentrifuge tubes	**MLS**
Mortar and pestle, rotor-stator homogenizer, or blunt needle and syringe	**MLS**
Tubes and other consumables	
Microcentrifuge tubes (1.5 mL) with screw caps	**MLS**
RNase-free pipette tips (sterile)	**MLS**
Disposable gloves	**MLS**
Ethanol, 96–100% (molecular biology grade)	**MLS**
Dithiothreitol (DTT, 2 M)	R0861
β-mercaptoethanol (14.3 M)	35602BID
PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)	**MLS**
TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA)	**MLS**
Lysozyme	**MLS**
Yeast lysis buffer (1 M sorbitol, 0.1 M EDTA, pH 7.4)	**MLS**
Lyticase or Zymolyase (20T)	**MLS**
(Optional) DNase I, RNase-free (1 U/µL)	EN0521
(Optional) RiboLock RNase Inhibitor	EO0381
(Optional) RapidOut DNA Removal Kit	K2981

Procedural guidelines

IMPORTANT! Tightly seal the bag containing purification columns after each use.

- Wear gloves when handling the Lysis Buffer and Wash Buffer I as these solutions contain irritants and are harmful if contacted with skin, inhaled, or swallowed.
- Unless otherwise indicated all purification steps are performed at room temperature (15–25°C).
- Keep the RNA on ice after extraction and while working with it.

Recommendations for avoiding RNA contamination

RNA purity and integrity is important 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 II and elution steps.

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

Recommendations for handling and storage of samples

- 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.

Before first use of the kit

1. Add the indicated volume of ethanol (96–100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) before first use.

Component	Cat. No. K0731 (50 reactions)		Cat. No. K0732 (250 reactions)	
Wash Buffer (concentrated)	**Wash Buffer I (40 mL)**	**Wash Buffer II (23 mL)**	**Wash Buffer I (200 mL)**	**Wash Buffer II (100 mL)**
Ethanol (96–100%)	10 mL	39 mL	50 mL	170 mL

2. Mark the checkbox on the bottlecap to indicate that ethanol has been added to the bottle

Before each use of the kit

Prepare reagents as required for procedures described below.

Prepare Lysis Buffer

1. Check Lysis Buffer for salt precipitation before each use. Redissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.

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

Prepare Proteinase K solution

Proteinase K solution is used for processing mammalian and insect tissue samples, and yeast samples. 600 µL of Proteinase K solution is required for each sample.
Add 10 µL of Proteinase K to 590 µL of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

(*For bacteria*) Prepare TE buffer

TE buffer with 0.4 mg/mL (final concentration) of lysozyme is used for processing gram-positive and gram-negative bacteria. 100 µL of TE buffer with lysozyme is required for each sample.
Add 0.4 mg of lysozyme to 1 mL of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA).

(*For yeast*) Prepare Yeast lysis buffer

Yeast lysis buffer is used for purifying total RNA from yesast. 100 µL of Yeast lysis buffer is required for each sample.
Add 0.1% β-mercaptoethanol and 50 units of lyticase or zymolyase 20T to Yeast lysis buffer (1 M sorbitol, 0.1 M EDTA, pH 7.4) prior to use.

Purify RNA from mammalian or insect tissue

Grind up to 30 mg of fresh or frozen tissue using a mortar and pestle or rotor-stator homogenizer.

Prepare tissue using a mortar and pestle

1. Place up to 30 mg of fresh or frozen tissue (use up to 10 mg of spleen tissue) into liquid nitrogen in a mortar, then grind thoroughly using a pestle.
2. Immediately transfer the powder into a 1.5 mL microcentrifuge tube containing 300 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT (see page 8).

   IMPORTANT!
   - Transfer the tissue or insect powder to the Lysis Buffer as quickly as possible. Leaving the powder without with the Lysis Buffer can result in degraded RNA.
   - All ground material must be thoroughly mixed with the Lysis Buffer and should not left dry on the walls of the tube (this can cause degradation of RNA).
   - The ground tissue or insect should be directly used for RNA purification and should not be stored.
3. Vortex for 10 seconds to mix thoroughly.
4. Homogenize the lysate using a rotor-stator homogenizer or pass the lysate through a blunt 20-gauge needle fitted to an RNase-free syringe several times.
5. Transfer lysate to a 1.5 mL RNase-free microcentrifuge tube.

Prepare tissue using a rotor-stator homogenizer

1. Place up to 30 mg of tissue (use up to 10 mg of spleen tissue) into a suitably sized vessel for homogenization containing 300 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT.
2. Immediately disrupt the material using a conventional rotor-stator homogenizer for 20–40 seconds or until the suspension is uniform.
3. Transfer lysate to a 1.5 mL RNase-free microcentrifuge tube.

Purify RNA from mammalian or insect tissue

1. Add 600 µL of Proteinase K solution (see page 8) to the 1.5 mL microcentrifuge tube containing homogenized lysate.
2. Vortex to mix thoroughly, then incubate at 15–25°C for 10 minutes.
3. Centrifuge at ≥12,000 × *g* for 5 minutes (if lysate is prepared from <10 mg of starting material) or 10 minutes (if lysate is prepared from >10 mg of starting material). Transfer the supernatant into a new RNase-free microcentrifuge tube.
4. Add 450 µL of ethanol (96–100%), then mix by pipetting.

5. Transfer up to 700 µL of lysate to a purification column inserted in a collection tube.

   Note: Close the bag with purification columns tightly after each use.

6. Centrifuge the column at ≥12,000 × g for 1 minute. Discard the flowthrough, then place the purification column back into the collection tube.

7. Repeat step 5 and step 6 until all of the lysate has been transferred into the column and centrifuged.

8. Discard the collection tube containing the flow-through solution. Place the purification column into a new 2 mL collection tube.

9. Add 700 µL of Wash Buffer I supplemented with ethanol (see page 8) to the purification column.

10. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

11. Add 600 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.

12. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

13. Add 250 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.

14. Centrifuge at ≥12,000 × *g* for 2 minutes.

15. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, place the purification column back into the collection tube, then centrifuge at maximum speed for 1 minute.

16. Discard the flow-through, then transfer the purification column to a sterile 1.5 mL RNase-free microcentrifuge tube.

17. Add 100 µL of nuclease-free water to the center of the purification column membrane. Centrifuge at ≥12,000 × *g* for 1 minute to elute RNA. Discard the purification column.

    More than 90% of RNA is eluted during the first elution step.

18. (*Optional*) Perform a second elution step with 100 µL of nuclease-free water for maximum RNA yield.

    Note: If more concentrated RNA is required, or the RNA is isolated from a small amount of starting material (For example <5 mg of tissue) the volume of nuclease-free water can be reduced to 50 µL for the first and second elution steps. Smaller volumes of eluant may result in a smaller final quantity of eluted RNA.

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

Purify RNA from mammalian culture cells

Prepare suspension cells

1. Pellet up to 1 × 10^7 cells in an appropriate centrifuge tube by centrifugation at 250 × *g* for 5 minutes. Discard the supernatant.
2. Rinse the cells once with PBS to remove residual growth medium.
3. Centrifuge at 250 × *g* for 5 minutes, then discard the supernatant.

Prepare adherent cells

1. Remove the growth medium from the cells (use up to 5 × 10^6 cells).
2. Rinse the cells once with PBS to remove residual medium. Remove, then discard PBS.
3. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization.
4. Transfer the cells into a microcentrifuge tube, then pellet the cells by centrifugation at 250 × *g* for 5 minutes. Discard the supernatant.

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

Purify RNA from mammalian cultured cells

1. Resuspend the cells in 600 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT (see page 8). Vortex for 10 seconds to mix thoroughly.
   a. If the mixture becomes viscous and dense after Lysis Buffer is added, homogenize the lysate for 30 seconds 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 results in a significant reduction of RNA yields.

   b. If any cell debris is observed in the sample, centrifuge the tubes at 14,000 × *g* for 5 minutes, then transfer the supernatant into a new RNase-free microcentrifuge tube.
2. Add 360 µL of ethanol (96–100%), then mix the sample by pipetting.
3. Transfer up to 700 µL of lysate to a purification column inserted in a collection tube.

   Note: Close the bag with purification columns tightly after each use.
4. Centrifuge the column at for ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
5. Repeat step 3 and step 4 until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution.

6. Place the purification column into a new 2 mL collection tube.
7. Add 700 µL of Wash Buffer I supplemented with ethanol (see page 8) to the purification column, then centrifuge at ≥12,000 × *g* for 1 minute.
8. Discard the flow-through, then place the purification column back into the collection tube.
9. Add 600 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column, then centrifuge at ≥12,000 × *g* for 1 minute.
10. Discard the flow-through, then place the purification column back into the collection tube.
11. Add 250 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column, then centrifuge at ≥12,000 × *g* for 2 minutes.
12. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, place the purification column back into the collection tube, then centrifuge at maximum speed for 1 minute.
13. Discard the collection tube containing the flow-through solution, then transfer the purification column to a sterile 1.5 mL RNase-free microcentrifuge tube.
14. Add 100 µL of nuclease-free water to the center of the purification column membrane. Centrifuge at ≥12,000 × *g* for 1 minute to elute RNA. Discard the purification column.

    More than 90% of RNA is eluted during the first elution step.
15. (*Optional*) Perform a second elution step with 100 µL of nuclease-free water for maximum RNA yield.

    If more concentrated RNA is required, or the RNA is isolated from a small amount of starting material (For example ≤1 × 10^6 cells) the volume of nuclease-free water can be reduced to 50 µL for the first and second elution steps. Smaller volumes of eluant may result in a smaller final quantity of eluted RNA.

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

Purify RNA from human blood cells

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.

1. Collect blood cells by centrifugation of 0.5 mL of whole blood at 400 × *g* for 5 minutes at 4ºC.

   Note: Blood cells will generate a pellet of approximately 60–70% of the total sample volume.

2. Remove the clear supernatant (plasma) from the pellet with a pipette.
3. Resuspend the pellet in 600 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT (see page 8). Vortex or pipet to mix thoroughly.
4. Add 450 µL of ethanol (96–100%), then mix by pipetting.
5. Transfer up to 700 µL of lysate to a purification column inserted in a collection tube.

   Note: Close the bag with purification columns tightly after each use.

6. Centrifuge the column at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
7. Repeat step 5 and step 6 until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the purification column into a new 2 mL collection tube
8. Add 700 µL of Wash Buffer I supplemented with ethanol (see page 8) to the purification column.
9. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
10. Add 600 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.
11. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
12. Add 250 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.
13. Centrifuge at ≥12,000 × *g* for 2 minutes.
14. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, place the purification column back into the collection tube, then centrifuge at maximum speed for 1 minute.
15. Discard the flow-through, then transfer the purification column to a sterile 1.5 mL RNase-free microcentrifuge tube.
16. Add 50 µL of nuclease-free water to the center of the purification column membrane. Centrifuge at ≥12,000 × *g* for 1 minute 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.

Purify RNA from bacteria

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

1. Transfer 1.5 mL of Gram-negative or Gram-positive bacterial culture (up to 1 × 10^9 cells) to a 1.5 mL microcentrifuge tube.
2. Collect cells by centrifugation at ≥12,000 x *g* for 2 minutes. Carefully remove the supernatant, leaving the pellet as dry as possible.
3. Resuspend the pellet in 100 µL of freshly prepared TE buffer supplemented with lysozyme (see page 8). Invert the tube several times to mix.
4. Incubate the resuspended cells for 15–25°C for 5 minutes.
5. Add 300 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT (see page 8). Mix thoroughly by vortexing for about 15 seconds until a homogeneous mixture is obtained.
6. Add 180 µL of ethanol (96–100%), then mix by pipetting.
7. Transfer up to 700 µL of lysate to a purification column inserted in a collection tube.

   Note: Close the bag with purification columns tightly after each use.

8. Centrifuge the column at ≥12,000 × g for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
9. Repeat step 7 and step 8 until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the purification column into a new 2 mL collection tube.
10. Add 700 µL of Wash Buffer I supplemented with ethanol (see page 8) to the purification column.
11. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
12. Add 600 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.
13. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
14. Add 250 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.
15. Centrifuge at ≥12,000 × *g* for 2 minutes.
16. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, place the purification column back into the collection tube, then centrifuge at maximum speed for 1 minute.
17. Discard the flow-through, then transfer the purification column to a sterile 1.5 mL RNase-free microcentrifuge tube.

18. Add 100 µL of nuclease-free water to the center of the purification column membrane. Centrifuge at ≥12,000 × *g* for 1 minute to elute RNA. Discard the purification column.

    More than 90% of RNA is eluted during the first elution step.

19. (*Optional*) Perform a second elution step with 100 µL of nuclease-free water for maximum RNA yield.

    If more concentrated RNA is required, or the RNA is isolated from a small amount of starting material (For example ≤1 × 10^6 cells) the volume of nuclease-free water can be reduced to 50 µL for the first and second elution steps. Smaller volumes of eluant may result in a smaller final quantity of eluted RNA.

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

Purify RNA from yeast

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

1. Transfer the yeast cell culture (up to 4 × 10^8 yeast cells) to a 1.5 mL microcentrifuge tube.
2. Collect the cells by centrifugation at ≥12,000 x *g* for 2 minutes. Discard the supernatant.
3. Resuspend the cell pellet in 100 µL of Yeast lysis buffer (see page 8). Incubate at 30°C for 30 minutes.
4. Add 300 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT (see page 8). Mix thoroughly by vortexing or pipetting.
5. Add 600 µL of Proteinase K solution (see page 8). Vortex to mix thoroughly, then incubate at 15–25°C for 10 minutes.
6. Centrifuge at ≥12,000 x *g* for 10 minutes. Transfer the supernatant into a new RNase-free microcentrifuge tube.
7. Add 450 µL of ethanol (96–100%), then mix by pipetting.
8. Transfer up to 700 µL of lysate to a purification column inserted in a collection tube.

   Note: Close the bag with purification columns tightly after each use.

9. Centrifuge the column at ≥12,000 × g for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
10. Repeat step 8 and step 9 until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the purification column into a new 2 mL collection tube
11. Add 700 µL of Wash Buffer I supplemented with ethanol (see page 8) to the purification column.

12. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

13. Add 600 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.

14. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.

15. Add 250 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.

16. Centrifuge at ≥12,000 × *g* for 2 minutes.

17. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, place the purification column back into the collection tube, then centrifuge at maximum speed for 1 minute.

18. Discard the flow-through, then transfer the purification column to a sterile 1.5 mL RNase-free microcentrifuge tube.

19. Add 100 µL of nuclease-free water to the center of the purification column membrane. Centrifuge at ≥12,000 × *g* for 1 minute to elute RNA. Discard the purification column.

    More than 90% of RNA is eluted during the first elution step.

20. (*Optional*) Perform a second elution step with 100 µL of nuclease-free water for maximum RNA yield.

Note: If more concentrated RNA is required, or the RNA is isolated from a small amount of starting material the volume of nuclease-free water can be reduced to 50 µL for the first and second elution steps. Smaller volumes of eluant may result in a smaller final quantity of eluted RNA.

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

Remove genomic DNA from RNA preparations

Remove genomic DNA from RNA preparations using DNase I

1. Add the following reagents to an RNase-free tube.

Reagent	Amount
RNA [1]	1 µg
10X reaction buffer with $MgCl_2$	1 µL
DNase I, RNase-free (1 U/µL) [2]	1 µL
(*Optional*) RiboLock RNase Inhibitor (40 U/µL) [3]	0.25 µL
Water, nuclease-free	to 10 µL

[1] The recommended final concentration of RNA is 0.1 µg/µL.
[2] Do not use more than 1 u of DNase I per 1 µg of RNA.
[3] RiboLock RNase Inhibitor can be added at 1 U/µL to prevent RNA degradation.

Note: Reaction volumes can be scaled to accommodate larger amounts of DNA or when working with diluted RNA samples, however, it is important to maintain the 1 u DNase/µg RNA ratio.

2. Incubate at 37°C for 30 minutes.

3. Add 1 µL 50 mM EDTA, then incubate at 65°C for 10 minutes to inactivate the DNase I.
   EDTA is required because RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent.
   When scaling reaction volumes, it is important to maintain the ~5 mM final EDTA concentration.

4. (*Optional*) Remove salts and enzymes from the RNA using the GeneJET™ RNA Purification Kit (see “Remove genomic DNA from RNA preparations using a kit” on page 17), or perform phenol/chloroform extraction.

Use the prepared RNA directly for downstream applications.

Remove genomic DNA from RNA preparations using a kit

The RapidOut DNA Removal Kit is designed for convenient removal of gDNA from RNA sample and subsequent removal of DNase I in a simple two-step procedure. The kit contains recombinant DNase I, RNase-free, and a proprietary DNase Removal Reagent for efficient DNase I removal. For ordering information see “Required materials not supplied” on page 6.

Perform RNA cleanup

The GeneJET™ RNA Purification Kit can be used to purify up to 100 µg of RNA after DNase I digestion or other enzymatic reactions.

1. Bring the volume of the reaction mixture to 100 µL with nuclease-free water.
2. Add 300 µL of Lysis Buffer without β-mercaptoethanol or DTT. Mix thoroughly by vortexing or pipetting.
3. Add 180 µL of ethanol (96–100%), then mix by pipetting.
4. Transfer the mixture to a purification column inserted in a collection tube. Centrifuge the column at ≥12,000 × *g* for 1 minute. Discard the flowthrough, then place the purification column into a new 2 mL collection tube

   Note: Close the bag with purification columns tightly after each use.

5. Add 700 µL of Wash Buffer I supplemented with ethanol (see page 8) to the purification column.
6. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
7. Add 600 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.
8. Centrifuge at ≥12,000 × *g* for 1 minute. Discard the flow-through, then place the purification column back into the collection tube.
9. Add 250 µL of Wash Buffer II supplemented with ethanol (see page 8) to the purification column.
10. Centrifuge at ≥12,000 × *g* for 2 minutes.
11. *(Optional)* If residual solution is seen in the purification column, empty the collection tube, place the purification column back into the collection tube, then centrifuge at maximum speed for 1 minute.
12. Discard the flow-through, then transfer the purification column to a sterile 1.5 mL RNase-free microcentrifuge tube.
13. Add 50 µL of nuclease-free water to the center of the purification column membrane. Centrifuge at ≥12,000 × *g* for 1 minute to elute RNA. Discard the purification column.

    Note: For maximum RNA yield repeat the elution step with an additional 50 µL of nuclease-free water.

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

Troubleshooting

Observation	Possible cause	Recommended action
Low RNA yield	Too much starting material was used for lysate preparation.	Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocol.
	Starting material was not completely disrupted.	Reduce the amount of starting material. Increase disruption time.
	Ethanol was not added to the lysate.	Ensure ethanol was added to the lysate before applying the sample to the purification column.
	Ethanol was not added to Wash Buffers.	Ensure ethanol was added to Wash Buffer I and Wash Buffer II before use. Follow the instructions for Wash Buffer preparation.
Degraded RNA	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.
	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.
DNA contamination	DNA was contaminated.	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.
Column clogging	Too much starting material was used for lysate preparation.	Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocol.
	Starting material was not completely disrupted.	Reduce the amount of starting material and increase disruption time.
	Tissue debris was not removed before applying lysate on a column.	Centrifuge the disrupted and homogenized cell suspension to remove tissue debris prior to applying the lysate to the column.
Enzymatic reactions not running well	Ethanol was carried over into the eluted RNA.	If residual solution is seen in the purification column after washing with Wash Buffer II, empty the collection tube, then centrifuge the column at ≥12,000 × *g* for 1 minute.
	Salt was carried over into the eluted RNA.	Use the Wash Buffers the correct order. Always wash the purification column with Wash Buffer I first, then proceed to wash with Wash Buffer II.

Limited product warranty

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