

Micro Sample Total RNA Extraction Kit (DNase I)

Cat. No.: T1521420 | Pack size: 50T | Storage: Store at 2-8°C, Room temperature, Store at -20°C

Product Information

Item	Description
Specifications & Purity	BioReagent, Suitable for molecular biology
Application	RNA Extraction
Storage Conditions	Store at 2-8°C, Room temperature, Store at -20°C
Shipped In	Ice chest + Ice pads
Stability And Storage	Each component has a shelf life of 15 months under corresponding storage conditions. T1521420A, T1521420B, T1521420C, T1521420D, T1521420E, T1521420F, T1521420G, T1521420H: Store at room temperature for 15 months. T1521420I, T1521420J, T1521420K: Store at 2-8°C for 15 months. T1521420L: Can be stored at 4°C for 1 month; long-term store at -20°C. Buffer URL containing β-mercaptoethanol can be stored at 4°C for 1 month.

Product Description

This product uses new technology to extract RNA from micro amounts of tissues and cells (as few as 10 cells). Special components added to the lysis buffer greatly increase the selective binding ability of RNA to the silica membrane. During the RNA extraction process, DNase I is added to completely remove trace DNA impurities, while DNase I and other residual substances are removed in subsequent washing steps, effectively ensuring RNA yield and purity.

Compared with ordinary RNA extraction methods, the Micro Sample RNA Extraction Kit selectively removes all RNA <200 bp (5.8S rRNA, 5S rRNA, tRNAs, etc.), while RNA >200 bp is enriched, separated, and purified. In this manual, different sample types have different procedures. The differences mainly lie in sample lysis and binding conditions to the silica membrane. Once RNA binds to the membrane, the subsequent steps are essentially similar.

Contents & Storage

T1521420	Component	50T	Storage
T1521420A	Buffer URL	30 mL	RT.
T1521420B	Buffer RW	38 mL	RT.
T1521420C	Buffer RW2	12 mL	RT.
T1521420D	RNase-Free ddH ₂ O	15 mL	RT.
T1521420E	Proteinase K	0.7 mL	RT.
T1521420F	Spin Column RM (with 2 mL Collection Tube)	1 EA×50	RT.
T1521420G	RNase-Free Centrifuge Tube (1.5 mL)	1 EA×50	RT.
T1521420H	1 mL Syringe	1 EA	RT.
T1521420I	DNase I	1 EA	2-8°C.
T1521420J	DNase I Buffer	4 mL	2-8°C.
T1521420K	RNase-Free ddH ₂ O	1 mL	2-8°C.
T1521420L	Trace RNA Enhancer	280 µL	-20°C.

T1521420A, T1521420B, T1521420C, T1521420D, T1521420E, T1521420F, T1521420G, T1521420H: Store at room temperature for 15 months.

T1521420I, T1521420J, T1521420K: Store at 2-8°C for 15 months.

T1521420L: Can be stored at 4°C for 1 month; long-term store at -20°C.

Buffer URL containing β-mercaptoethanol can be stored at 4°C for 1 month.

Precautions

1. Reagents and consumables to be prepared by the user:

β-mercaptoethanol, absolute ethanol, 1.5 mL RNase-Free centrifuge tubes.

2. Determining sample amount

To obtain high yield and purity using this kit, appropriate sample amount is important because the binding capacity of the spin column and the volume of lysis buffer are limited. Only when the sample is fully lysed and RNA is adequately bound to the silica membrane can the RNA yield be ensured.

Table 1. Technical specifications of the Micro Sample RNA Extraction Kit

Parameter	Value
Maximum spin column capacity	700 μ L
RNA size extracted	>200 bp
Minimum elution volume	10 μ L
Maximum sample amount (animal cells)	5×10^5
Maximum sample amount (animal tissue)	5 mg

Note: If the maximum binding capacity of the kit is exceeded, RNA yield may decrease. If the sample is not completely lysed, RNA yield will also decrease.

Table 2. Number of HeLa cells under different culture conditions

Culture Vessel	Growth Area (cm ²)	Cell Number
96-well plate	0.32-0.6	$4-5 \times 10^4$
48-well plate	1	1×10^5
24-well plate	2	2.5×10^5
12-well plate	4	5×10^5
6-well plate	9.5	1×10^6
Dish (35 mm)	8	1×10^6
Flask (40-50 mL)	25	3×10^6

3. Sample storage and handling

RNA in untreated tissue is unprotected and easily degraded. Fresh tissue should be quickly frozen in liquid nitrogen and stored immediately at -70°C . Frozen tissue should not be repeatedly freeze-thawed to avoid RNA degradation. Samples can also be homogenized, added to Buffer URL, and stored at -70°C ; frozen samples can be stored stably for several months.

Pre-operation Precautions

To prevent RNA degradation, avoid leaving untreated samples at room temperature for extended periods. Before tissue fixation, RNA is unprotected, so freeze the tissue in liquid nitrogen.

Tissue stored in Buffer URL can be stored at -70°C for several months. When processing frozen lysates, thaw samples completely at room temperature or in a 37°C water bath. Ensure that the salt in the lysate is completely dissolved, then proceed immediately (prolonged 37°C treatment may cause chemical degradation of RNA). Before use, prepare 70% ethanol using RNase-Free ddH₂O.

Preparation of DNase I stock solution: Use a 1 mL syringe to inject 550 μ L RNase-Free ddH₂O into the vial containing DNase I powder. Mix gently until dissolved. Aliquot and store at -30 to -15°C (can be stored for 9 months). Thaw aliquots as needed. The prepared DNase I working solution can be stored at 2-8°C for up to 42 days. Avoid repeated freeze-thaw cycles.

Protocol

Preparation before use: Add 48 mL of absolute ethanol to 12 mL of Buffer RW2.

Total RNA Extraction from Formalin-Fixed Microdissected Samples When extracting RNA from formalin-fixed microdissected samples, the main influencing factors are sample age, fixation process, and storage conditions. RNA may be degraded into fragments <300 bp. This kit efficiently binds fragments >200 bp, so if the RNA in the sample is highly degraded, extraction may fail.

1. Add an appropriate volume of Buffer URL (add β -mercaptoethanol to a final concentration of 1% before use) to the sample. The volume of Buffer URL added depends on the capacity of the microdissection instrument, but should not exceed 140 μ L. If needed, transfer the sample and Buffer URL to a 1.5 mL or 2 mL RNase-Free centrifuge tube (user-supplied). Add Buffer URL to bring the total volume to 150 μ L.

Note: When processing less than 5000 cells, add 5 μ L Trace RNA Enhancer to the solution.

2. Add 295 μ L RNase-Free ddH₂O to the solution, then add 5 μ L Proteinase K. Mix with a pipette and incubate at 55°C for 10 minutes. Centrifuge at room temperature at 12,000 rpm (13,680 $\times$ g) for 3 minutes.

Note: Tissue debris may form a pellet, and sometimes a thin film may float on top of the solution.

3. Transfer the upper solution (approximately 450 μ L) to a new RNase-Free centrifuge tube (user-supplied) using a pipette.

Note: When aspirating the upper solution, be careful to avoid touching the tissue debris pellet. Insert the pipette tip below the film to avoid transferring the film to the new tube.

4. Add 0.5 volumes of absolute ethanol (usually 225 μ L) to the transferred upper solution and mix with a pipette. Transfer all solution and precipitate to a Spin Column RM (placed in a collection tube). Centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 seconds. Discard the flow-through and return the column to the collection tube.

Note: After adding ethanol, a precipitate may appear, but this does not affect RNA extraction.

5. Add 350 μ L Buffer RW to the column. Centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 seconds. Discard the flow-through and return the column to the collection tube.

6. Preparation of DNase I working solution: Transfer 10 μ L of DNase I stock solution to a new RNase-Free centrifuge tube, add 70 μ L DNase I Buffer, and mix gently.

7. Add 80 μL of the DNase I working solution to the center of the column. Let stand at room temperature for 15 minutes.
8. Add 350 μL Buffer RW to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.
9. Add 500 μL Buffer RW2 (ensure absolute ethanol has been added before use) to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm ($13,680 \times g$) for 2 minutes.
12. Transfer the column to a new 1.5 mL RNase-Free centrifuge tube. Open the lid and let stand at room temperature for 1 minute to completely air-dry any residual ethanol. Add 10–15 μL RNase-Free ddH₂O to the center of the membrane. Let stand at room temperature for 1 minute, then centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds to collect the RNA solution.

Note: The elution volume should not be less than 10 μL , otherwise total RNA yield may be affected.

Total RNA Extraction from Animal Tissue

The following procedure is suitable for RNA extraction from most animal tissues. For fibrous tissues, refer to Section III (Total RNA Extraction from Fibrous Tissue). For high-purity and high-yield RNA from animal tissue, the starting amount is critical. The lysis buffer can lyse up to 5 mg of tissue. Some tissues (spleen, certain brain regions, lung, thymus) may form precipitates during extraction, but this does not affect RNA extraction.

Recommended tissue amount: ≤ 5 mg. Do not exceed the binding capacity of the Spin Column RM, otherwise RNA yield and purity may decrease.

1. Determine the total tissue amount (not exceeding 5 mg) and proceed immediately to step 2.
2. Homogenize (2a) or grind (2b) the tissue piece in Buffer URL (add β -mercaptoethanol to a final concentration of 1% before use).

Note: When processing less than 10 μg of tissue, add 5 μL Trace RNA Enhancer to the lysis buffer. Incomplete homogenization may lead to low RNA yield and column clogging.

(2a) Homogenization using a homogenizer:

Place the weighed tissue piece into a suitable container, add 350 μL Buffer URL, and immediately homogenize until a uniform solution is obtained (typically 20–40 seconds). Proceed to step 3.

(2b) Grinding using a pestle:

Add 350 μL Buffer URL to a 1.5 mL RNase-Free centrifuge tube (user-supplied). Quickly transfer the tissue piece from -80°C into the tube. Grind thoroughly with a pestle to completely disrupt the tissue, avoiding freeze-thaw. Transfer the solution to a Filter Column GS (user-supplied, placed in a

collection tube). Centrifuge at 12,000 rpm ($13,680 \times g$) for 2 minutes. Collect the flow-through. Proceed to step 3.

Note: When using a pestle, ensure the tissue is completely ground to achieve full lysis.

3. Centrifuge the homogenized sample (2a) or filtrate (2b) at 12,000 rpm ($13,680 \times g$) for 3 minutes. Carefully transfer the supernatant to a new 1.5 mL RNase-Free centrifuge tube (user-supplied) using a pipette.

4. Add 1 volume (350 μ L) of 70% ethanol to the supernatant and mix with a pipette. Transfer all solution and precipitate to a Spin Column RM (placed in a collection tube). Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.

Note: If some lysis buffer was lost during previous steps, reduce the amount of 70% ethanol accordingly. After adding ethanol, a precipitate may appear, but this does not affect RNA extraction.

5. Add 350 μ L Buffer RW to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.

6. Preparation of DNase I working solution: Transfer 10 μ L of DNase I stock solution to a new RNase-Free centrifuge tube, add 70 μ L DNase I Buffer, and mix gently.

7. Add 80 μ L of the DNase I working solution to the center of the column. Let stand at room temperature for 15 minutes.

8. Add 350 μ L Buffer RW to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.

9. Add 500 μ L Buffer RW2 (ensure absolute ethanol has been added before use) to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.

10. Repeat step 9.

11. Centrifuge at 12,000 rpm ($13,680 \times g$) for 2 minutes.

12. Transfer the column to a new 1.5 mL RNase-Free centrifuge tube. Open the lid and let stand at room temperature for 1 minute to completely air-dry any residual ethanol. Add 10–15 μ L RNase-Free ddH₂O to the center of the membrane. Let stand at room temperature for 1 minute, then centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds to collect the RNA solution.

Note: The elution volume should not be less than 10 μ L, otherwise total RNA yield may be affected.

Total RNA Extraction from Fibrous Tissue

Fibrous tissues (e.g., skeletal muscle, heart, skin) contain large amounts of contractile proteins, connective tissue, and collagen. Removal of these proteins is necessary for successful RNA extraction. Therefore, for total RNA extraction from fibrous tissue, a Proteinase K digestion step is introduced.

The following procedure can successfully extract RNA from heart, muscle, and skin tissues. Other protein-rich tissues can also use this method. However, the buffer used during Proteinase K digestion

does not provide long-term effective RNase inhibition, so this method is not suitable for RNA extraction from RNase-rich tissues such as spleen and intestine.

Recommended tissue amount: ≤ 5 mg. Do not exceed the binding capacity of the spin column, otherwise RNA yield and purity may decrease.

1. Determine the total tissue amount (not exceeding 5 mg) and proceed immediately to step 2.
2. Homogenize (2a) or grind (2b) the tissue piece in Buffer URL (add β -mercaptoethanol to a final concentration of 1% before use).

Note: When processing less than 10 μ g of tissue, add 5 μ L Trace RNA Enhancer to the lysis buffer. Incomplete homogenization may lead to low RNA yield and column clogging. Using a homogenizer or filter column yields higher RNA recovery than other methods.

(2a) Homogenization using a homogenizer:

Place the weighed tissue piece into a suitable container, add 150 μ L Buffer URL, and immediately homogenize until a uniform solution is obtained (typically 20–40 seconds). Proceed to step 3.

(2b) Grinding using a pestle:

Add 150 μ L Buffer URL to a 1.5 mL RNase-Free centrifuge tube (user-supplied). Quickly transfer the tissue piece from -80°C into the tube. Grind thoroughly with a pestle to completely disrupt the tissue, avoiding freeze-thaw. Proceed to step 3.

Note: When using a pestle, ensure the tissue is completely ground to achieve full lysis.

3. Add 295 μ L RNase-Free ddH₂O to the solution, then add 5 μ L Proteinase K. Mix with a pipette. Incubate at 55°C for 10 minutes. Centrifuge at room temperature at 12,000 rpm ($13,680 \times g$) for 3 minutes.

Note: Tissue debris may form a pellet, and sometimes a thin film may float on top of the solution.

4. Transfer the upper solution (usually about 450 μ L) to a new 1.5 mL RNase-Free centrifuge tube (user-supplied) using a pipette.

Note: When aspirating the upper solution, be careful to avoid touching the tissue debris pellet. Insert the pipette tip below the film to avoid transferring the film to the new tube.

5. Add 0.5 volumes of absolute ethanol (usually 225 μ L) to the transferred upper solution and mix with a pipette. Transfer all solution and precipitate to a Spin Column RM (placed in a collection tube). Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.

Note: After adding ethanol, a precipitate may appear, but this does not affect RNA extraction.

6. Add 350 μ L Buffer RW to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.

7. Preparation of DNase I working solution: Transfer 10 μL of DNase I stock solution to a new RNase-Free centrifuge tube, add 70 μL DNase I Buffer, and mix gently.
8. Add 80 μL of the DNase I working solution to the center of the column. Let stand at room temperature for 15 minutes.
9. Add 350 μL Buffer RW to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.
10. Add 500 μL Buffer RW2 (ensure absolute ethanol has been added before use) to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.
11. Repeat step 10.
12. Centrifuge at 12,000 rpm ($13,680 \times g$) for 2 minutes.
13. Transfer the column to a new 1.5 mL RNase-Free centrifuge tube. Open the lid and let stand at room temperature for 1 minute to completely air-dry any residual ethanol. Add 10–15 μL RNase-Free ddH₂O to the center of the membrane. Let stand at room temperature for 1 minute, then centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds to collect the RNA solution.

Note: The elution volume should not be less than 10 μL , otherwise total RNA yield may be affected.

Total RNA Extraction from Animal Cells

Recommended cell amount: $\leq 5 \times 10^5$. Do not exceed the binding capacity of the spin column, otherwise RNA yield and purity may decrease.

1. Cell processing and lysis

1.1 Cell pellet

Gently tap the bottom of the tube to disperse the cells. Add 350 μL Buffer URL (add β -mercaptoethanol to a final concentration of 1% before use). Vortex or mix with a pipette. Proceed to step 2.

1.2 Suspension culture cells

Determine the cell number (see Table 2). Pellet the cells by centrifugation at $300 \times g$ for 5 minutes in an RNase-Free centrifuge tube (user-supplied). Remove all supernatant. Add 350 μL Buffer URL (add β -mercaptoethanol to a final concentration of 1% before use). Proceed to step 2.

1.3 Monolayer adherent cells

These cells can be collected directly in the culture vessel (maximum diameter 10 cm), or they can be trypsinized and then pelleted for lysis.

Direct lysis in the culture vessel:

Determine the cell number. Completely remove the culture medium. Add 350 μL Buffer URL (add β -mercaptoethanol to a final concentration of 1% before use) to lyse the cells. Collect the cell lysate

and transfer to a 1.5 mL RNase-Free centrifuge tube (user-supplied). Vortex or mix with a pipette until no cell clumps are visible. Proceed to step 2.

Trypsinization (for monolayer adherent cells grown in flasks):

Determine the cell number. Aspirate the medium, wash cells with PBS, aspirate PBS, and treat cells with PBS containing 0.10%–0.25% trypsin. When cells detach from the vessel wall, add serum-containing medium to inactivate trypsin. Transfer cells to an RNase-Free centrifuge tube. Pellet cells by centrifugation at 300×g for 5 minutes. Carefully remove all supernatant. Add 350 µL Buffer URL (add β-mercaptoethanol to a final concentration of 1% before use) to lyse the cells. Proceed to step 2.

Notes:

- ① If culture medium is not completely removed, it will dilute the lysis buffer, affecting lysis efficiency and RNA binding to the silica membrane, leading to reduced RNA yield.
- ② When processing $\leq 1 \times 10^5$ cells, the volume of Buffer URL can be reduced to 75 µL. Use a smaller centrifuge tube and vortex for 1 minute to homogenize the lysate. Then proceed to step 2.
- ③ If processing <5000 cells, add 5 µL Trace RNA Enhancer to the lysis buffer.
- ④ Incomplete resuspension of cells will lead to insufficient lysis and reduced RNA yield.

2. Sample homogenization (see steps 2a, 2b)

Note: Incomplete homogenization may lead to low RNA yield and clogging of the Spin Column CR1. Using a homogenizer or filter column yields higher RNA recovery than other methods.

(2a) Transfer the cell lysate to a Filter Column GS (user-supplied, placed in a collection tube). Centrifuge at 12,000 rpm (13,680 × g) for 2 minutes. Collect the flow-through. Proceed to step 3.

(2b) Homogenize using a homogenizer for 30 seconds.

3. Add 1 volume (usually 350 µL) of 70% ethanol and mix with a pipette. Transfer all solution and precipitate to a Spin Column RM (placed in a collection tube). Centrifuge at 12,000 rpm (13,680 × g) for 30–60 seconds. Discard the flow-through and return the column to the collection tube.

Note: If some lysis buffer was lost during homogenization, reduce the amount of 70% ethanol accordingly. If only 75 µL of Buffer URL was added in step 2, then add only 75 µL of 70% ethanol here. After adding ethanol, a precipitate may appear, but this does not affect RNA extraction.

4. Add 350 µL Buffer RW to the column. Centrifuge at 12,000 rpm (13,680 × g) for 30 seconds. Discard the flow-through and return the column to the collection tube.

5. Preparation of DNase I working solution: Transfer 10 µL of DNase I stock solution to a new RNase-Free centrifuge tube, add 70 µL DNase I Buffer, and mix gently.

6. Add 80 µL of the DNase I working solution to the center of the column. Let stand at room temperature for 15 minutes.

7. Add 350 μL Buffer RW to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.
8. Add 500 μL Buffer RW2 (ensure absolute ethanol has been added before use) to the column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds. Discard the flow-through and return the column to the collection tube.
9. Repeat step 8.
10. Centrifuge at 12,000 rpm ($13,680 \times g$) for 2 minutes.
11. Transfer the column to a new 1.5 mL RNase-Free centrifuge tube. Open the lid and let stand at room temperature for 1 minute to completely air-dry any residual ethanol. Add 10–15 μL RNase-Free ddH₂O to the center of the membrane. Let stand at room temperature for 1 minute, then centrifuge at 12,000 rpm ($13,680 \times g$) for 30 seconds to collect the RNA solution.

Note: The elution volume should not be less than 10 μL , otherwise total RNA yield may be affected.

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