



EZ-10 RNA Mini-Preps Kits Handbook



- EZ-10 Total RNA Mini-Preps Kit
- RNase-Free DNase Set
- EZ-10 DNAaway RNA Mini-Preps Kit



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EZ-10 Total RNA Mini-Preps Kit

■ Component

Component	BS88583, 50 Preps	BS88584, 100 Preps	BS88586, 250 Preps
Buffer RLT	20 ml	40 ml	100 ml
DW Solution (concentrate)	36 ml	72 ml	180 ml
RPE Solution (concentrate)	12 ml	24 ml	60 ml
RNase-Free Water	35 ml	70 ml	175 ml
EZ-10 Spin Column	50	100	250
2 ml Collection Tube	50	100	250
Proteinase K	0.6 ml	1.2 ml	3 ml
Protocol	1	1	1

■ Storage

Buffer RLT should be stored at 4°C and the others are stored at room temperature. The kit is stable for 1 year under these conditions.

Proteinase K solution is stable at room temperature for 6 months, store at 2-8°C for long term.

■ Notes

Before using for the first time, add **4 ml/8 ml/20 ml** ethanol to **36 ml/72 ml/180 ml** DW solution and **48 ml/96 ml/240 ml** ethanol to **12 ml/24 ml/60 ml** RPE solution to make a working solution.

	BS88583, 50 Preps	BS88584, 100 Preps	BS88586, 250 Preps
DW Solution	36 ml	72 ml	180 ml
Ethanol (96-100%)	4 ml	8 ml	20 ml
DW Final Volume	40 ml	80 ml	200 ml



	BS88583, 50 Preps	BS88584, 100 Preps	BS88586, 250 Preps
RPE Solution	12 ml	24 ml	60 ml
Ethanol (96-100%)	48 ml	96 ml	240 ml
RPE Final Volume	60 ml	120 ml	300 ml

Salt precipitation in Buffer RLT and DW Solution (concentrate) may occur at low temperature. Redissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.

Care must be taken when working with RNA. It is important to maintain an RNase-free environment starting with RNA sample preparation and continue through purification and analysis. Use RNase free tubes, tips, gels. Wear gloves at all the time.

■ Quality Control

Each lot of EZ-10 Total RNA Mini-Preps Kit is tested against predetermined specifications to ensure consistent product quality.

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC
RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

■ Introduction

The EZ-10 Total RNA Mini-Preps Kit allows efficient purification of total RNA from various samples, total RNA is easily purified from animal or human cells and tissues using a simple spin format.

Biological samples are first lysed and homogenized in denaturing guanidine-containing buffer (Buffer RLT), which immediately inactivates DNases and RNases as well as proteases to ensure isolation of intact RNA. RNA in the whole homogenate is selectively absorbed on EZ-10 Spin Column, all protein and other components are removed in the flow-through. Remaining contaminants and salts are efficiently washed away. Purified RNA is eluted in RNase-free water has OD₂₆₀/OD₂₈₀ ratios of 1.9-2.1 (measured in 10 mM Tris-Cl, pH 7.5) and is ideal for use in most downstream applications including Northern blotting, RT-PCR, Quantitative PCR, Poly (A) RNA selection and Array analysis.

■ Features

1. Fast. Using a rapid spin column format, the whole procedure takes less than 20 minutes.
2. High Quality of RNA. Purified RNA has an OD₂₆₀/OD₂₈₀ ratio of 1.9-2.0.
3. Versatile. Suitable for purification of total RNA from various samples such as bacteria, fungi, animals and some of plants.
4. Economic.

■ Materials Supplied by User

Microcentrifuge capable of at least 12,000 × *g*
RNase-free pipets, pipet tips and vortexer
RNase-free microcentrifuge tubes (1.5 ml or 2 ml)
Ethanol (96-100%)
RNase-free water

Lysozyme solution:

400 µg/ml lysozyme in RNase-free water for Gram-negative bacterium

3 mg/ml lysozyme in RNase-free water for Gram-positive bacterium

■ Protocol

1. Sample preparation

A. Adherent cells: Discard the culture medium, add 0.3 ml Buffer RLT for approximate 4 cm² cultured cell, mix gently by pipetting.

B. Suspension cells: Collect cells by centrifugation, discard the supernatant and add 0.3 ml Buffer RLT for 1 × 10⁶ cells, mix gently by pipetting.

✓ The amount of cells should not exceed 1 × 10⁶ for fibroblasts or carcinoma cell.

C. Animal tissue:

a. Cut the tissue into pieces and grind to fine powder in liquid nitrogen, add 0.3 ml Buffer RLT for 10-20 mg tissue.

✓ For tissues with a very high number of cells, such as spleen, no more than 10 mg starting material should be used.
 ✓ Do not let the tissue sample thaw before you add the Buffer RLT.

b. Mix thoroughly by vortexing, and incubate at room temperature for 5 minutes.

✓ After incubation the lysate may appear viscous, but should not be gelatinous. If the lysate appears gelatinous, mix it by vigorous shaking.

c. Add 0.59 ml RNase-free water and 10 µl proteinase K. Mix thoroughly and incubate at 55°C for 10 minutes.

d. Centrifuge at 12,000 × g for 3 minutes at 4°C. Transfer the supernatant to a new RNase-free 1.5 ml centrifuge tube.

D. Plant tissue:

a. Cut the tissue into pieces and grind to fine powder in liquid nitrogen, add 0.3 ml Buffer RLT for 25 mg plant tissue. Mix thoroughly by vortexing, and incubate at room temperature for 5 minutes.

✓ Do not let the plant sample thaw before you add the Buffer RLT

b. Centrifuge at 12,000 × g for 3 minutes at 4°C. Transfer the supernatant to a new RNase-free 1.5 ml centrifuge tube.

E. Gram-negative bacterium:

a. Transfer 1 ml logarithmic phase culture (about 2 × 10⁹ cells) into centrifuge tube and centrifuge at 10,000 × g for 30 seconds, discard supernatant.

b. Add 100 µl lysozyme solution (400 µg/ml lysozyme in RNase-free water. NOT supplied in the kit), suspend thoroughly and incubate at 37°C for 5 minutes.

c. Add 0.3 ml Buffer RLT, mix thoroughly by vortexing, and incubate at room temperature for 5 minutes.

F. Gram-positive bacterium:

a. Transfer 1 ml logarithmic phase culture (about 2 × 10⁹ cells) into centrifuge tube and centrifuge at 10,000 × g for 30 seconds, discard supernatant.

b. Add 100 µl lysozyme solution (3 mg/ml lysozyme in RNase-free water. NOT supplied in the kit), suspend thoroughly and incubate at 37°C for 10 minutes.

c. Add 0.3 ml Buffer RLT, mix thoroughly by vortexing, and incubate at room temperature for 5 minutes.

2. Add 1/2 volume of ethanol, mix by inverting the tube.

3. Place the EZ-10 Spin Column in a 2 ml collection tube. Transfer the mixture from step 2 to the spin column, centrifuge at 12,000 × g for 2 minutes at room temperature, discard the flow-through.

✓ If the sample volume exceeds 0.7 ml, centrifuge successive aliquots in the same spin column. Make sure that no liquid remains on the column membrane after centrifugation.

4. Place the EZ-10 Spin Column in the collection tube, and add 0.35 ml of DW Solution to the column, centrifuge at 12,000 × g for 1 minute at room temperature, discard the flow-through.

✓ Check the label to ensure DW Solution was diluted with ethanol.
 ✓ If RNase-Free DNase Set (BS88254) is used, start BS88254 after completion of step 4.

5. Add 0.5 ml of RPE Solution to the column, centrifuge at 12,000 × g for 1 minute at room temperature, discard the flow-through.

✓ Check the label to ensure RPE Solution was diluted with ethanol.

6. Repeat step 5 once.

7. Centrifuge the column at 12,000 × g for 2 minutes at room temperature. Incubate the open column at room temperature for 2 minutes until the ethanol has evaporated.

✓ This step is very important to remove the residual ethanol.
 ✓ Residual ethanol may interfere with subsequent reactions.

8. Place the column in a new RNase-free 1.5 ml centrifuge tube, add 50 µl RNase-free water, keep at room temperature for 2 minutes.

**9. Centrifuge at 12,000 × g for 1 minute at room temperature.**

- ✓ The solution in the centrifuge tube is the RNA sample, it can be used immediately for downstream molecular operation or stored at -70°C.
- ✓ For certain tissues with extremely high DNA content (e.g. thymus). Try using RNase-Free DNase Set (BS88254) to removal of genomic DNA.

■ Troubleshooting Guide**1. Clumping in binding steps**

- A. Make sure samples are homogenized completely before binding the spin column.
- B. Reduce the amount of starting material.
- C. For plant or animal tissues, centrifuge at 12,000 × g for 3 minutes at 4°C and transfer the supernatant to a new 1.5 ml RNase-free centrifuge tube before adding ethanol.

2. RNA degradation

- A. Use fresh sample. For frozen samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at -70°C.
- B. We recommend treating the tissue samples with liquid nitrogen and proceeding to sample lysis immediately.
- C. Create a RNase-free working environment.
- D. Wear gloves during all steps of the procedure. Change gloves frequently.
- E. Use of RNase-free microcentrifuge tubes is recommended.

3. Low yield

- A. Homogenize tissue completely.
- B. Use advisable amount of starting material.
- C. Check the label to ensure DW Solution and RPE Solution were diluted with ethanol.
- D. Elution step has to be strictly followed.

4. Inhibition of downstream enzymatic reactions

- A. Residual ethanol from the DW or RPE Solution can inhibit downstream enzymatic reactions. Centrifuge the column at 12,000 × g for 2 minutes to remove the residual ethanol.

5. RNA is contaminated with DNA

- A. Reduce the amount of starting material.
- B. For certain tissues with extremely high DNA content (e.g. *thymus*), using RNase-Free DNase Set (BS88254) to remove of genomic DNA.
- C. Completely removal of cell-culture medium or stabilization reagent.

RNase-Free DNase Set**■ Component**

Component	BS88253, 50 Preps	BS88254, 100 Preps
RNase-Free DNase I (1 U/μl)	1500 μl	3000 μl
Buffer RDD	3 ml	6 ml
RNase-Free Water	2 ml	4 ml
Protocol	1	1

■ Storage

DNase I should be stored at -20°C and the others are stored at 4°C. The kit is stable for 9 months under these conditions.

■ Note

Buffer RDD is optimized for on-column DNase digestion. The buffer is also well-suited for efficient DNase digestion in solution.

The RNase-Free DNase I is supplied as liquid form. You can divide it into single-use aliquots, and store at -20°C for up to 9 months. Do not refreeze the aliquots after thawing.

Care must be taken when working with RNA. It is important to maintain an RNase-free environment starting with RNA sample preparation and continue through purification and analysis. Use RNase-free tubes, tips, gels. Wear gloves at all the time.

■ Quality Control

Each lot of RNase-Free DNase Set is tested against predetermined specifications to ensure consistent product quality.

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■ Introduction

In some cases, purification RNA contains genomic DNA which may interfere with subsequent reactions such as transcription analysis, cDNA synthesis, Q-RT PCR, and Northern Analysis.

RNase-Free DNase I Set provides efficient on-column digestion of DNA during RNA purification using EZ-10 Total RNA Mini-Preps Kit. Genomic DNA can be completely removed by DNase I treatment. Then the DNase I is efficiently removed by DW Solution. Purified RNA is suitable for cDNA synthesis, Q-RT PCR, mRNA isolation, probe generation, Northern blot analysis, primer extension, RNA protection assay and *In vitro* translation. The procedure is simple and fast, requires no need for phenol/chloroform extractions. The whole procedure takes less than 40 minutes.

■ Features

1. Provides efficient on-column digestion of DNA during RNA purification.
2. The kit can be compatible with EZ-10 Total RNA Mini-Preps Kit.
3. The entire procedure can be completed in 40 minutes.
4. Completely removal of DNA contaminants.
5. High yield and reproducibility.
6. No phenol/chloroform extraction or ethanol precipitation required.

■ Materials Supplied by User

Microcentrifuge capable of at least 12,000 × *g*
RNase-free pipets and pipet tips
RNase-free microcentrifuge tubes (1.5 ml or 2 ml)
Ethanol (96-100%)

■ Protocol

1. Prepare DNase I work solution:

Add 10 µl RNase-free Water and 40 µl Buffer RDD to 1.5 ml RNase-free centrifuge tube. Add 30 µl DNase I (30 U) to the mixture above, mix gently by inverting the tube. Put the tube on ice for next step.

✓ Avoid mixing the work solution by vortexer. DNase I is especially sensitive to physical denaturation.

2. Removal of DNA is compatible with EZ-10 Total RNA Mini-Preps Kit.

After completion of step 4 on page 5, add 80 µl DNase I work solution to the center of the membrane. Keep at 25-37°C for 20-30 minutes.

✓ The optimal reaction temperature is 25-37°C.

3. Add 0.35 ml of DW Solution to the column, keep at room temperature for 3 minutes.

✓ This step is to remove DNase I.
✓ Check the label to ensure DW Solution was diluted with ethanol.

4. Centrifuge at 12,000 × *g* for 1 minute at room temperature, discard the flow-through.

5. Continue the step 5-9 on page 5-6.

✓ It is also compatible with other Column Total RNA Mini-Preps Kit.

6. Purified RNA is ready for use. For long term storage, keep at -70°C.



■ Troubleshooting Guide

1. DNA contamination remains after the initial treatment

- Choose the optimal reaction temperature (25-37°C) to treat genomic DNA.
- Do not refreeze the DNase solution after thawing.
- Avoid mixing the work solution by vortexer. DNase I is especially sensitive to physical denaturation.
- Reduce the amount of starting material.
- You may extend the DNase I digest time.

2. RNA degradation

- Divide the DNase I in RNase-free environment.
- Use fresh sample. For frozen samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at -70°C.
- We recommend treating the tissue samples with liquid nitrogen and proceeding to sample lysis immediately.
- Wear gloves during all steps of the procedure. Change gloves frequently.
- Use of RNase-free microcentrifuge tubes is recommended.

3. Inhibition of downstream reactions

- Removal of DNase I and wash step has to be strictly followed.
- Residual ethanol from the DW or RPE Solution can inhibit downstream enzymatic reactions. Centrifuge the column at 12,000 × *g* for 2 minutes to remove the residual ethanol.

EZ-10 DNAaway RNA Mini-Preps Kit

■ Component

Component	BS88133, 50 Preps	BS88136, 250 Preps
Buffer Lysis-DR	20 ml	100 ml
GT Solution (concentrate)	18 ml	90 ml
NT Solution (concentrate)	6 ml	30 ml
gDNA Eliminator Column (with 2 ml Collection Tube)	50	250
RZ-10 RNA Column (with 2 ml Collection Tube)	50	250
RNase-Free Water	5 ml	25 ml
Protocol	1	1

■ Storage

The kit should be stored dry, at room temperature (15-25°C) and are stable for 1 year under these conditions.

■ Note

Salt precipitation in Buffer Lysis-DR and GT Solution (concentrate) may occur at low temperature. Redissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.

GT Solution and NT Solution are supplied as concentrates. Before using for the first time, add **12/60 ml** ethanol to **18/90 ml** GT solution and **24/120 ml** ethanol to **6/30 ml** NT solution to make a working solution.

	BS88133, 50 Preps	BS88136, 250 Preps
GT Solution	18 ml	90 ml
Ethanol (96-100%)	12 ml	60 ml
GT Final Volume	30 ml	150 ml



	BS88133, 50 Preps	BS88136, 250 Preps
NT Solution	6 ml	30 ml
Ethanol (96-100%)	24 ml	120 ml
NT Final Volume	30 ml	150 ml

Care must be taken when working with RNA. It is important to maintain an RNase-free environment starting with RNA sample preparation and continue through purification and analysis. Use RNase free tubes, tips, gels. Wear gloves at all the time.

■ Quality Control

Each lot of EZ-10 DNAaway RNA Mini-Preps Kit is tested against predetermined specifications to ensure consistent product quality.

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■ Introduction

The EZ-10 DNAaway RNA Mini-Preps Kit is designed to purify RNA from small amounts of animal cells or tissues. Samples are lysed and homogenized by Buffer Lysis-DR. Genomic DNA contamination is effectively removed using a specially gDNA Eliminator Column. RNA is absorbed on RZ-10 RNA Column. Finally, the RNA is eluted from RZ-10 RNA Column. The purified RNA is ready to use and is ideally suited for downstream applications that are sensitive to low amounts of DNA contamination, such as quantitative, real-time RT-PCR. The procedure is simple and fast, requires no need for phenol/chloroform extraction. The whole procedure takes less than 30 minutes.

■ Features

1. Provides efficient gDNA Eliminator Column to remove DNA during RNA purification.
2. Completely removal of DNA contaminant.
3. The entire procedure can be completed in 30 minutes.
4. High yield and reproducible.
5. No phenol/chloroform extraction or ethanol precipitation required.
6. High-purity RNA is suited for downstream applications that are sensitive to low amounts of DNA contamination.

■ Materials Supplied by User

Microcentrifuge capable of at least 12,000 × *g*
RNase-free pipets and pipet tips
RNase-free microcentrifuge tubes (1.5 ml or 2 ml)
Vortexer
Ethanol (96-100%)

■ Protocol

1. Sample Preparation

A. Cell Cultures

1a. Cells grown in suspension: Spin appropriate number of cells (max. 1×10^7) at $300 \times g$ for 5 minutes at room temperature. Remove supernatant carefully, proceed to step 2.

1b. Cells grown in monolayer: Aspirate the medium and add 350 μ l Buffer Lysis-DR to the cell-culture dish. Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube. Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

- ✓ If sample can not be used immediately for genomic DNA extraction, it is recommended to store at -80°C for long-term.
- ✓ Avoid repeated freezing and thawing of stored samples, as this leads to RNA degradation.

B. Animal tissue: Grind 15~30 mg animal tissue to fine powder in liquid nitrogen. Transfer the powder to 1.5 ml RNase-free centrifuge tube.

C. Plant : Grind 25~50 mg plant tissue to fine powder in liquid nitrogen. Transfer the powder to 1.5 ml RNase-free centrifuge tube.

2. Add 350 μ l Buffer Lysis-DR immediately to the 1.5 ml RNase-free centrifuge tube above, mix by vortex.

- ✓ Incubate the mixture for 5 minutes at room temperature.

3. Place the gDNA Eliminator Column in a 2 ml collection tube. Transfer the lysate to the column, keep at room temperature for 1 minute, centrifuge at $9,000 \times g$ for 1 minute at room temperature.

4. Transfer the flow-through to a new RNase-free tube for RNA purification.

5. Add 250 μ l ethanol to the flow-through from step 4, mix thoroughly.

6. Place the RZ-10 RNA Column in the collection tube and transfer the mixture from step 5 to the RZ-10 RNA Column, centrifuge at $9,000 \times g$ for 1 minute at room temperature.

7. Place the RZ-10 RNA Column in the collection tube, add 500 μ l GT Solution, keep at room temperature for 1 minute, centrifuge at $9,000 \times g$ for 1 minute at room temperature. Discard the flow-through.

- ✓ Check the label to ensure GT Solution was diluted with ethanol.

8. Add 500 μ l NT Solution to the column, keep at room temperature for 1 minute. Followed by centrifuge at $9,000 \times g$ for 1 minute at room temperature. Discard the flow-through.

- ✓ Check the label to ensure NT Solution was diluted with ethanol.

9. Place the column in the collection tube, and centrifuge at $9,000 \times g$ for 2 minutes at room temperature.

10. Transfer the column into a new RNase-free centrifuge tube, and open the lid until the ethanol has completely evaporated (about 3-5 minutes).

- ✓ It is important to dry the membrane of the RZ-10 RNA Column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

11. Add 30-50 μ l RNase-free water, keep at room temperature for 2 minutes, and centrifuge at $9,000 \times g$ for 2 minutes.

- ✓ The solution in the centrifuge tube is the RNA sample, it can be used immediately for downstream molecular operation or stored at -70°C .

12. Purified RNA is ready to use, keep at -80°C for long term.



■ Troubleshooting Guide

1. Clumping in binding steps

- Make sure samples are homogenized completely before binding the spin column.
- Reduce the amount of starting material.

2. RNA degradation

- Use fresh sample. For frozen samples, ensure that they were frozen immediately in liquid nitrogen and properly stored at -70°C.
- We recommend treating the tissue samples with liquid nitrogen and proceeding to sample lysis immediately.
- Create a RNase-free working environment.
- Wear gloves during all steps of the procedure. Change gloves frequently.
- Using of RNase-free microcentrifuge tubes is recommended.

3. Low yield

- Homogenize tissue completely.
- Use advisable amount of starting material.
- Check the label to ensure GT Solution and NT Solution were diluted with ethanol.
- Elution step has to be strictly followed.

4. Inhibition of downstream enzymatic reactions

- Residual ethanol from GT Solution and NT Solution can inhibit downstream enzymatic reactions. Centrifuge the column at 12,000 × g for 2 minutes to remove the residual ethanol thoroughly.
- Residual salt can inhibit downstream enzymatic reactions. Ensure that GT Solution, NT Solution have been used at room temperature (15–25°C).

5. DNA residual

- Reduce the amount of starting material.
- Completely removal of cell-culture medium or stabilization reagent.

■ Ordering Information

Code	Product Name	Size
BS88583	EZ-10 Total RNA Mini-Preps Kit	50 Preps
BS88584	EZ-10 Total RNA Mini-Preps Kit	100 Preps
BS88586	EZ-10 Total RNA Mini-Preps Kit	250 Preps
BS88253	RNase-Free DNase Set	50 Preps
BS88254	RNase-Free DNase Set	100 Preps
BS88133	EZ-10 DNAaway RNA Mini-Preps Kit	50 Preps
BS88136	EZ-10 DNAaway RNA Mini-Preps Kit	250 Preps

■ Related Products

Code	Product Name	Size
BS410A	EZ-RNA Reagents	100 ml
DB0154	DEPC	100 ml
LDB0308	Lysozyme	5 g
SD5008	EZ-10 Spin Column	100
BS88203	All-In-One DNA/RNA Mini-Preps Kit	50 Preps
BS88003	All-In-One DNA/RNA/Protein Mini-Preps Kit	50 Preps
BS91915	EZ-10 Spin Column Fungal RNA Mini-Preps Kit	50 Preps
BS82314	EZ-10 Spin Column Plant RNA Mini-Preps Kit	50 Preps
BS82312	EZ-10 Spin Column Animal Total RNA Extraction Kit	50 Preps
BS82313	EZ-10 Spin Column Blood RNA Mini-Preps Kit	50 Preps
MT91928	mRNA Purification Kit from Total RNA	25 Preps