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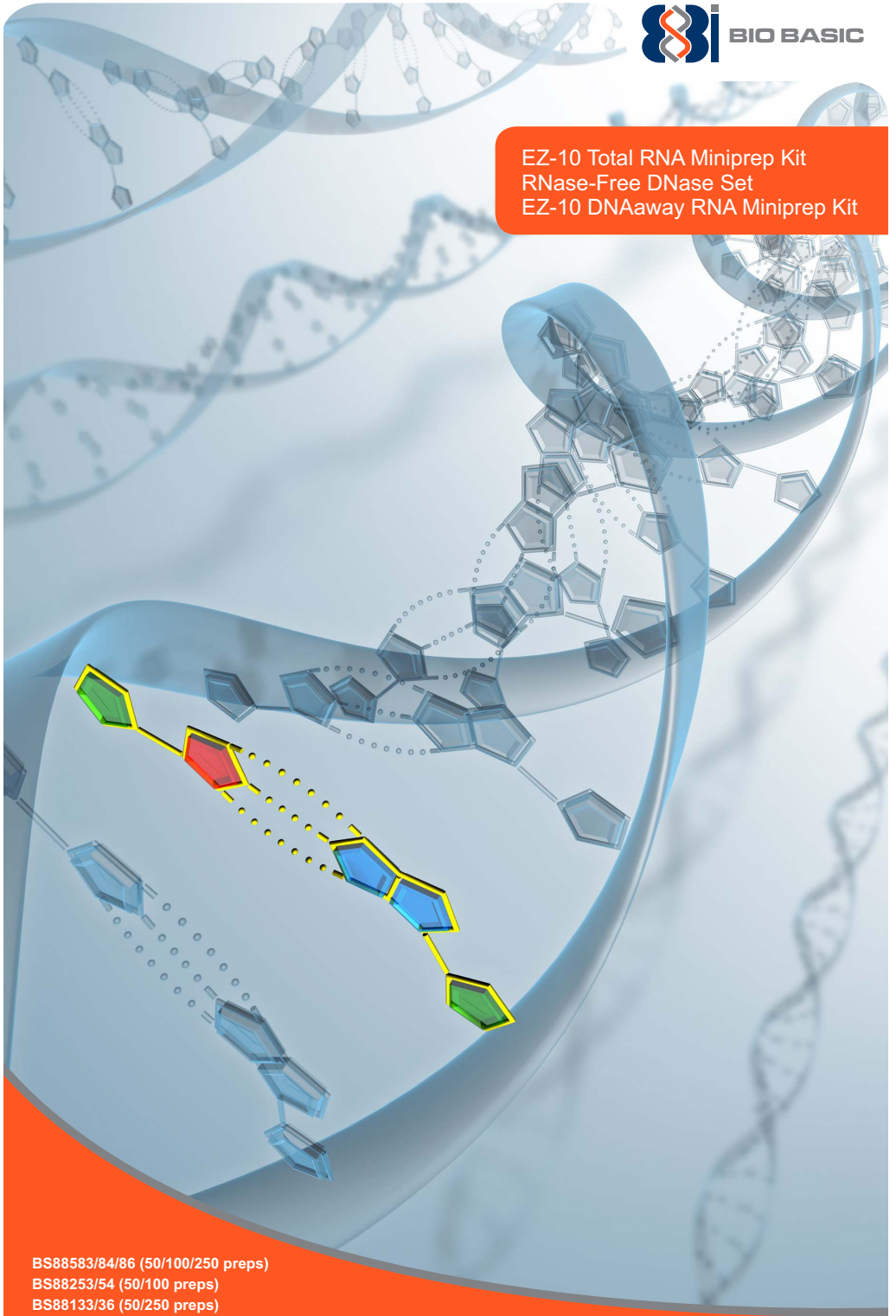
EZ-10 Total RNA Miniprep Kit
RNase-Free DNase Set
EZ-10 DNAaway RNA Miniprep Kit



**BS88583/84/86 (50/100/250 preps)**
**BS88253/54 (50/100 preps)**
**BS88133/36 (50/250 preps)**


*QF 24 TV4*
*CV1 2021*


***For Research Use Only***

# EZ-10 RNA Miniprep Kit Handbook





# EZ-10 RNA Miniprep Kits (BS88583, BS88584, BS88586)

## Kit Contents

| 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 | 30 ml | 60 ml | 150 ml |
| EZ-10 Spin Column | 50 | 100 | 250 |
| 2 ml Collection Tube | 50 | 100 | 250 |
| Proteinase K | 600 µl | 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 the first use, depending on your purchased kit:

• add **4ml** / **8ml** / **20ml** ethanol to **36ml** / **72ml** / **180ml DW solution** respectively:

| Component | 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 |

• also add **48ml** / **96ml** / **240ml** ethanol to **12ml** / **24ml** / **60ml RPE solution** respectively to make a working solution:

| Component | 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 isimportant 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 Minipreps Kit is tested against predetermined specifications to ensure consistent product quality.

## 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 and is ideal for use in most downstream applications including Northern blotting, RT-PCR, Quantitative PCR, Poly (A) RNA selection and Array analysis.

## Features

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

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 cm^2 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 × $10^6$ cells, mix gently by pipetting.
• The amount of cells should not exceed 1 × $10^6$ 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 x $10^9$ cells) into centrifuge tube and centrifuge at 10,000 x 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 x $10^9$ cells) into centrifuge tube and centrifuge at 10,000 x 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.

**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 0.7ml, centrifuge successive aliquots in the same spin column. Make sure 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) for 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), use Rnase-Free DNase Set (BS88254) to remove of genomic DNA.
C. Complete removal of cell-culture medium or stabilization reagent.

# RNase-Free Dnase Set (BS88253, BS88254)

## Kit Contents

| 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 should be stored at -20°C; Please refer to the label of other components for their respective storage conditions. The kit is stable for 9 months under these conditions.

### Notes

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.

## 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

- Provides efficient on-column digestion of DNA during RNA purification.
- The kit can be compatible with EZ-10 Total RNA Miniprep Kit.
- The entire procedure can be completed in 40 minutes.
- Complete removal of DNA contaminants.
- High yield and reproducibility.
- No phenol/chloroform extraction or ethanol precipitation required.

## Materials Supplied by User

- Microcentrifuge capable of at least 12,000 × g
- RNase-free pipettes and pipette 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 Miniprep Kit.

After completion of step 4 of the EZ-10 Total RNA Minipreps Kit (BS88583, BS88584, BS88586), 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.

- The following steps below are the same as from steps 5-9 of the EZ-10 Total RNA Minipreps Kit (BS88583, BS88584, BS88586) This kit is also compatible with other column Total RNA Minipreps Kits

**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.

**10.** Purified RNA is ready for use. For long term storage, keep at -70°C. Try using RNase-Free DNase Set (BS88254) for removal of genomic DNA.

## Troubleshooting Guide

**1. DNA contamination remains after the initial treatment**

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

**2. RNA degradation**

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



**3. Inhibition of downstream reactions**

A. Removal of DNase I and wash step has to be strictly followed.
B. 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 Miniprep Kit (BS88133, BS88136)

## Kit Contents

| Component | BS88183, 50 Preps | BS885136, 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 |
| EZ-10 RNA Column (with 2-mi Collection Tube) | 50 | 250 |
| RNase-Free Water | 5 ml | 25ml |
| Protocol | 1 | 1 |

## Storage

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

### Notes

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 the first use, depending on your purchased kit:
add **12ml** / **60ml** ethanol to **18ml** / **90ml GT solution** respectively:

| Component | 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 |

Also add **24ml** / **120ml** ethanol to **6ml** / **30ml NT solution** respectively to make a working solution:

| Component | BS88133, 50 Preps | BS88136, 250 Preps |
|---|---|---|
| NT Solution | 18 ml | 90 ml |
| Ethanol (96-100%) | 12 ml | 60 ml |
| GT 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 Miniprep Kit is tested against predetermined specifications to ensure consistent product quality.

## 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 EZ-10 RNA Column. Finally, the RNA is eluted from EZ-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

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

## Materials Supplied by Users

- 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 x $10^7$) at 300 x 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 µl Buffer Lysis-DR to the cell-culture dish. Collect the lysate with a rubber policeman. Pipette the lysate into a microcentrifuge tube. Vortex or pipette to mix, and ensure that no cell clumps are visible before proceeding to step 2.

- If sample can not be used immediately for genomic DNA extraction, it is recommended to store at -80ºC 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 µl 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 x g for 1 minute at room temperature.

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

**5.** Add 250 µl ethanol to the flow-through from step 4, mix throughly.

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

**7.** Place the EZ-10 RNA Column in the collection tube, add 500 µl GT Solution, keep at room temperature for 1 minute, centrifuge at 9,000 x 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 µl NT Solution to the column, keep at room temperature for 1 minute. Followed by centrifuge at 9,000 x 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 x g for 2 minutes at room temperature.

**10.** Transfer the column into a new RNase-free centrifuge tube, and open it until the ethanol has completely evaporated (about 3-5 minutes).
- It is important to dry the membrane of the EZ-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 µl RNase-free water, keep at room temperature for 2 minutes, and centrifuge at 9,000 x 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 storage.

## 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.

**2. RNA degradation**

A. Use fresh sample. For frozen samples, ensure that they were 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 an 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 GT Solution and NT Solution were diluted with ethanol.
D. Elution step has to be strictly followed.

**4. Inhibition of downstream enzymatic reactions**

A. 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.
B. 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**

A. Reduce the amount of starting material.
B. Complete removal of cell-culture medium or stabilization reagent.

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NOT INTENDED FOR HUMAN OR ANIMAL USE