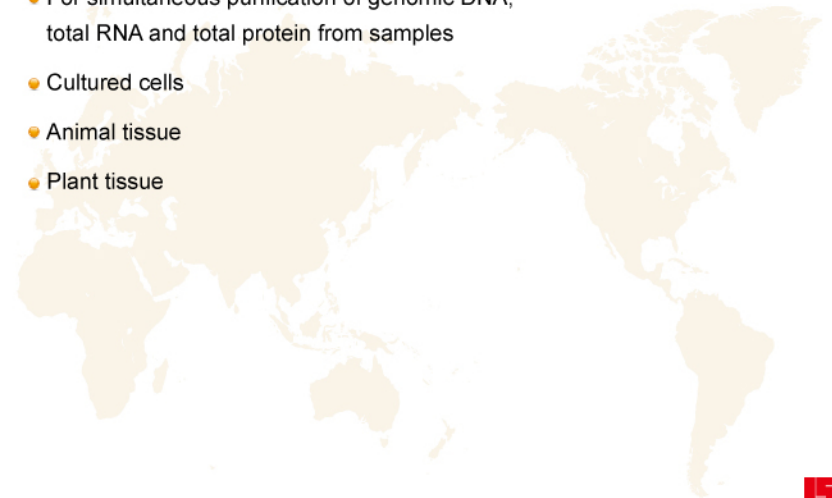




All-In-One Mini-Preps Kit Handbook (Cell, Tissue, Plant)



- For simultaneous purification of genomic DNA, total RNA and total protein from samples
- Cultured cells
- Animal tissue
- Plant tissue



BIO BASIC CANADA INC.

Add: 20 Konrad Cres. Markham ON L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com


www.biobasic.com

September 2011/Rev. 01

Contents

All-In-One DNA/RNA/Protein Mini-Preps Kit

Kit Contents	3
Storage	3
Introduction	4
Features	4
Applications	5
Quality Control	5
Materials Supplied by User	6
Before Starting	6
DNA/RNA/Protein Isolation Procedure	7
Protocol	8
Genomic DNA Purification	9
Total RNA Purification	10
Total Protein Precipitation	11
General Guidelines	12

All-In-One DNA/RNA Mini-Preps Kit

Kit Contents	13
Storage	13
Introduction	13
Features	14
Applications	14
Quality Control	15
Materials Supplied by User	15
Before Starting	15
DNA/RNA Isolation Procedure	16



Protocol	17
General Guidelines	20
Notes for Sample Preparation	21
Sample Collection and Storage	21
Sample Homogenize	21
Starting Amounts of Samples	21
Quantity and Quality of Genomic DNA	22
UV Spectrophotometric Analysis of DNA	22
Agarose gel electrophoresis	22
Quantity and Quality of Total RNA	23
Formaldehyde Agarose Gel Electrophoresis	23
Troubleshooting Guide	25
Appendix	27
The Parameter of Column	27
Centrifugation Speed	27
Precipitation of RNA with Ethanol	27
Ordering Information	28
Related Products	28

All-In-One DNA/RNA/Protein Mini-Preps Kit

■ Kit Contents

Component	BS88003, 50 Preps
Buffer Lysis-DRP	45 ml
CW1 Solution (concentrate)	13 ml
CW2 Solution (concentrate)	9 ml
CE Buffer	10 ml
GT Solution (concentrate)	18 ml
NT Solution (concentrate)	6 ml
RNase-Free Water	5 ml
PP Solution	35 ml
PD Solution	10 ml
EZ-10 DNA Column (blue) (with 2 ml Collection Tube)	50
RZ-10 RNA Column (colorless) (with 2 ml Collection Tube)	50
Protocol	1

■ Storage

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

■ Introduction

All-In-One DNA/RNA/Protein Mini-Preps Kit is designed for simultaneous extraction of total RNA, genomic DNA and protein from a single biological sample. DNA, RNA and protein are isolated without splitting the sample prior to extraction. DNA, RNA and Protein can be isolated from cultured eukaryotic cells, animal and plant tissues.

This kit provides an innovative buffer system and a silica-based column nucleic acid purification technology to separate pure genomic DNA from and total RNA. There is no need for phenol/chloroform extraction. Purified DNA is suitable for downstream applications such as Restriction Endonuclease Digestions, PCR and other applications. Isolated total RNA can be used for mRNA isolation, probe generation, RT-PCR, Northern blot analysis, primer extension, RNA protection assay and *In vitro* translation.

Protein is purified in denatured form with a special buffer (PP Solution) which effectively precipitates protein. After washing step, the protein pellet is dissolved in PD Solution. Isolated protein is suitable for SDS-PAGE, Western Blot analysis, and quantification. The procedure is simple and fast. Genomic DNA, total RNA and Proteins can be isolated in less than 1 hour.

■ Features

1. Genomic DNA, RNA and Proteins can be simultaneously isolated in less than 1 hour.
2. Preparation of high quality genomic DNA with a molecular weight \geq 20 kb.
3. Preparation of high quality total RNA without genomic DNA.
4. Isolated protein is suitable for SDS-PAGE and Western Blot analysis.
5. High yield and reproducibility.
6. No phenol/chloroform extraction or ethanol precipitation required.

■ Applications

Direct correlation between genetic and proteomic data.

Efficient of up to 10 μ g of genomic DNA purification from animal tissue. Purified genomic DNA has an average length of 20-30 kb. DNA of this length is particularly suitable for PCR, where complete denaturation of the template is important to achieve the highest amplification efficiency. The purified DNA is ready to use in any downstream applications, including:

- ▲ PCR and real-time PCR
- ▲ Southern, dot and slot blot analyses
- ▲ Comparative genome hybridization (CGH)
- ▲ Genotyping, SNP analysis

Efficient of up to 20 μ g of total RNA purification from animal tissue. The purified RNA is ready to use in any downstream applications, including:

- ▲ RT-PCR
- ▲ Quantitative, real-time RT-PCR
- ▲ Differential display
- ▲ cDNA synthesis
- ▲ Northern, dot, and slot blot analyses
- ▲ Primer extension
- ▲ Poly A + RNA selection
- ▲ RNase/S1 nuclease protection
- ▲ Microarrays

Isolation protein in denatured form. The purified protein is suitable for downstream applications such as:

- ▲ 1D gel electrophoresis
- ▲ Western blotting

■ Quality Control

Each lot of All-in-One DNA/RNA/Protein Mini-Preps Kit is tested against predetermined specifications to ensure consistent product quality.

Materials Supplied by User

Microcentrifuge capable of at least 12,000 × g

RNase-Free pipets and pipet tips

Vortexer

RNase-Free ethanol (96-100%)

50% Ethanol

RNase-Free microcentrifuge tubes (1.5 ml)

Before Starting

Check the Buffer Lysis-DRP for salt precipitation before each use. If necessary, redissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.

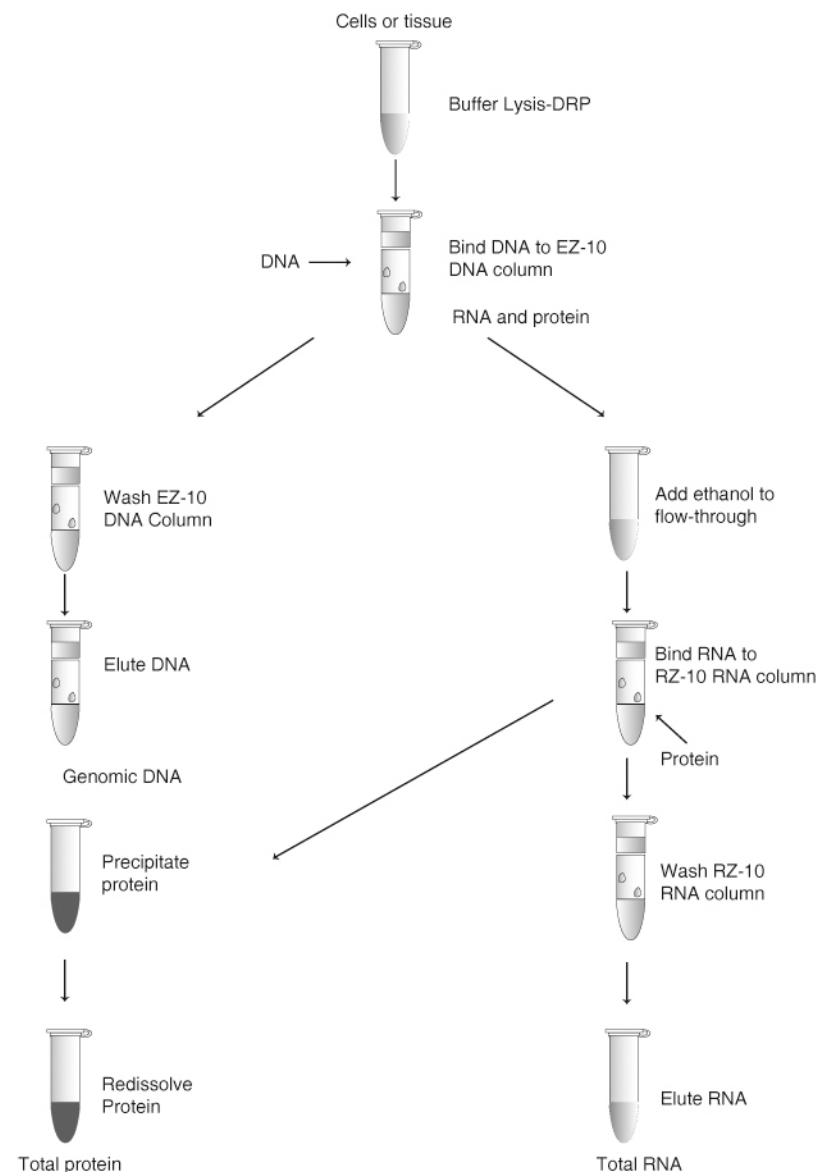
CE Buffer is 10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0. Water can be used as eluate in the DNA final step should EDTA be avoided for the following applications. However, this is not recommended if the pH of water is less than 7.0.

CW1 Solution, CW2 Solution, GT Solution and NT Solution are supplied as concentrates. Before using for the first time, add **17 ml** ethanol to **13 ml** CW1 Solution, **21 ml** ethanol to **9 ml** CW2 Solution, **12 ml** ethanol to **18 ml** GT solution and **24 ml** ethanol to **6 ml** NT solution to make a work solution.

	Ethanol (96-100%)	Final Volume
CW1 Solution (13 ml)	17 ml	30 ml
CW2 Solution (9 ml)	21 ml	30 ml
GT solution (18 ml)	12 ml	30 ml
NT solution (6 ml)	24 ml	30 ml

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

DNA/RNA/Protein Isolation Procedure



■ 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-DRP 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, since this leads to RNA degradation.

B. Animal tissue: Grind 15–30 mg animal tissue to fine powder in liquid nitrogen. Transfer the frozen powder to 1.5 ml RNase-free centrifuge tube, and allow the liquid nitrogen to evaporate.

- ✓ 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 adding Buffer Lysis-DRP.

C. Plant: Grind 25–50 mg plant tissue to fine powder in liquid nitrogen. Transfer the frozen powder to 1.5 ml RNase-free centrifuge tube, and allow the liquid nitrogen to evaporate.

- ✓ Do not let the tissue sample thaw before adding Buffer Lysis-DRP.

2. Add 350 μ l Buffer Lysis-DRP immediately to the 1.5 ml RNase-Free centrifuge tube above, mix by vortex. Incubate at room temperature for 5 minutes.

3. Centrifuge at $12,000 \times g$ for 3 minutes at 4°C . Transfer the supernatant to a new RNase-Free tube.

● Genomic DNA Purification

4. Place the EZ-10 DNA 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. Transfer the flow-through to a new RNase-Free tube for RNA purification.

- ✓ Store the flow-through at 4°C or isolation RNA (step 11-17) before DNA wash and elution.

5. Add 350 μ l Buffer Lysis-DRP to the EZ-10 DNA Column, keep at room temperature for 1 minute, centrifuge at $9,000 \times g$ for 1 minute at room temperature, discard the liquid in the collection tube.

6. Place the EZ-10 DNA Column in the collection tube, and add 500 μ l CW1 Solution to the EZ-10 DNA Column, keep at room temperature for 1 minute, centrifuge at $9,000 \times g$ for 1 minute at room temperature, discard the liquid in the collection tube.

- ✓ Check the label to ensure CW1 Solution was diluted with ethanol.

7. Place the EZ-10 DNA Column in the collection tube, and add 500 μ l CW2 Solution to the EZ-10 DNA Column, keep at room temperature for 1 minute, centrifuge at $9,000 \times g$ for 1 minute at room temperature, discard the liquid in the collection tube.

- ✓ Check the label to ensure CW2 Solution was diluted with ethanol.

8. Place the EZ-10 DNA Column in the collection tube, and centrifuge at $9,000 \times g$ for 2 minutes at room temperature.

9. Incubate the open spin column at room temperature for 2-3 minutes until the ethanol has completely evaporated. Transfer the spin column to a clean 1.5 ml centrifuge tube.

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

10. Add 50 μ l CE Buffer directly onto the center part of EZ-10 DNA Column membrane. Incubate at room temperature for 2 minutes, and then centrifuge for 2 minutes at $9,000 \times g$ to elute the DNA.

- ✓ Warm the Buffer CE to 60°C will increase the elution efficiency.
- ✓ Elution with more than 50 µl (e.g. 100 µl) increases the DNA yield, but the concentration will be lower.
- ✓ For maximum DNA yield, repeat elution once as described in this step.
- ✓ A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate.

● Total RNA Purification

NOTE: 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 times.

11. Add 250 µl ethanol to the flow-through from step 4, mix thoroughly.
12. Place the RZ-10 RNA Column in the collection tube and transfer mixture to the RZ-10 RNA Column, centrifuge at 9,000 x g for 1 minute at room temperature.
13. Transfer the flow-through to a new 1.5 ml centrifuge tube for total protein purification.
14. Place the RZ-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.
15. Add 500 µl NT Solution to the column, 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 NT Solution was diluted with ethanol.
16. Place the column in the collection tube, and centrifuge at 9,000 x g for 2 minutes at room temperature.

✓ 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.

17. Transfer the column into a new RNase-Free centrifuge tube, 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.

● Total Protein Precipitation

18. Add 600 µl PP Solution to the centrifuge tube from step 13, mix thoroughly, and keep at room temperature for 10 minutes to precipitate protein.
19. Centrifuge at 9,000 x g for 10 minutes at room temperature, and carefully discard the supernatant.
20. Add 500 µl 50% ethanol to the protein pellet, centrifuge at 9,000 x g for 1 minute, and remove the supernatant as much liquid as possible.

✓ When washing the protein pellet with 50% ethanol, disturb the pellet by pipetting up and down several times.
21. Dry the protein pellet for 5-10 minutes at room temperature.
22. Add up to 100 µl PD Solution and mix thoroughly to dissolve the protein pellet.
23. Incubate for 5 minutes at 95°C to completely dissolve and denature the protein. Then cool the sample to room temperature.
24. Centrifuge at 9,000 x g for 2 minutes to pellet any residual insoluble material.
25. Transfer the supernatant to a new 1.5 ml centrifuge tube.

✓ The solution in the centrifuge tube is the protein solution, it can be used immediately for downstream applications such as SDS-PAGE and western blotting or stored at -20°C.

■ General Guidelines

All-In-One DNA/RNA/Protein Mini-Preps Kit provides a new technology for selective binding of double-stranded DNA with EZ-10 DNA Column, and binding of total RNA with RZ-10 RNA Column, and purifying protein with a new protein precipitation chemistry.

Biological samples are first lysed and homogenized in a highly denaturing guanidine-containing buffer (buffer lysis-DRP), which immediately inactivates DNases and RNases as well as proteases to ensure isolation of intact DNA, RNA.

DNA in the whole homogenate is selectively absorbed on EZ-10 DNA Column. Wash the membrane with the buffer lysis-DRP to remove RNA/Protein contaminants on the EZ-10 DNA spin column. PCR inhibitors, protein and salts are completely removed with CW1 solution and CW2 solution. Purified genomic DNA is eluted in CE buffer.

Ethanol is added to the flow-through from the EZ-10 DNA spin column to provide appropriate binding conditions for RNA, and the sample is then applied to a RZ-10 spin column, where total RNA binds to the membrane. PCR inhibitors, protein and salts are completely removed with GT Solution and NT solution. Purified RNA is eluted in RNase-Free Water.

Buffer PP Solution, a novel aqueous protein precipitation solution, is added to the flow-through of the RZ-10 spin column, and the precipitated proteins are pelleted by centrifugation. Intact total proteins are redissolved in PD Solution and then ready to use in downstream applications.

All-In-One DNA/RNA Mini-Preps Kit

■ Kit Contents

Component	BS88203, 50 Preps
Buffer Lysis-DR	20 ml
CW1 Solution (concentrate)	13 ml
CW2 Solution (concentrate)	9 ml
CE Buffer	10 ml
GT Solution (concentrate)	18 ml
NT Solution (concentrate)	6 ml
RNase-Free Water	5 ml
EZ-10 DNA Column (blue) (with 2 ml Collection Tube)	50
RZ-10 RNA Column (colorless) (with 2 ml Collection Tube)	50
Protocol	1

■ Storage

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

■ Introduction

All-In-One DNA/RNA Mini-Preps Kit is designed for simultaneous extraction of total RNA, and genomic DNA from a single biological sample. DNA and RNA are isolated without splitting the sample prior to extraction. DNA and RNA can be isolated from cultured eukaryotic cells, animal and plant tissues.

This kit, using an innovative buffer system, no need for phenol/chloroform extractions and provides column to separately pure genomic DNA from total RNA. Purified DNA is suitable for downstream applications such as restriction endonuclease digestions, PCR, and so on. Isolated RNA can be used for mRNA isolation, probe generation, RT-PCR, northern blot analysis, primer extension, RNA protection assay and *In vitro* translation. The procedure is simple and fast. Genomic DNA and RNA can be isolated in less than 40 minutes.

■ Features

1. Genomic DNA and total RNA can be simultaneously isolated in less than 40 minutes.
2. Preparation of high quality genomic DNA with a molecular weight ≥ 20 kb.
3. Preparation of high quality total RNA without genomic DNA.
4. High yield and reproducibility.
5. No phenol/chloroform extraction or ethanol precipitation required.

■ Applications

Efficient of up to 10 μ g of genomic DNA purification from animal tissue. Purified genomic DNA has an average length of 20-30 kb. DNA of this length is particularly suitable for PCR, where complete denaturation of the template is important to achieve the highest amplification efficiency. The purified DNA is ready to use in any downstream applications, including:

- ▲ PCR and real-time PCR
- ▲ Southern, dot, and slot blot analyses
- ▲ Comparative genome hybridization (CGH)
- ▲ Genotyping, SNP analysis

Efficient of up to 20 μ g of total RNA purification from animal tissue. The purified RNA is ready to use in any downstream applications, including:

- ▲ RT-PCR
- ▲ Quantitative, real-time RT-PCR
- ▲ Differential display
- ▲ cDNA synthesis
- ▲ Northern, dot and slot blot analyses
- ▲ Primer extension
- ▲ Poly A + RNA selection
- ▲ RNase/S1 nuclease protection
- ▲ Microarrays

■ Quality Control

Each lot of All-In-One DNA/RNA Mini-Preps Kit is tested against predetermined specifications to ensure consistent product quality.

■ Materials Supplied by User

- Microcentrifuge capable of at least 12,000 $\times g$
- RNase-Free pipets and pipet tips
- Vortexer
- RNase-Free ethanol (96-100%)
- RNase-Free microcentrifuge tubes (1.5 ml)

■ Before Starting

Check the Buffer Lysis-DR for salt precipitation before each use. If necessary, redissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.

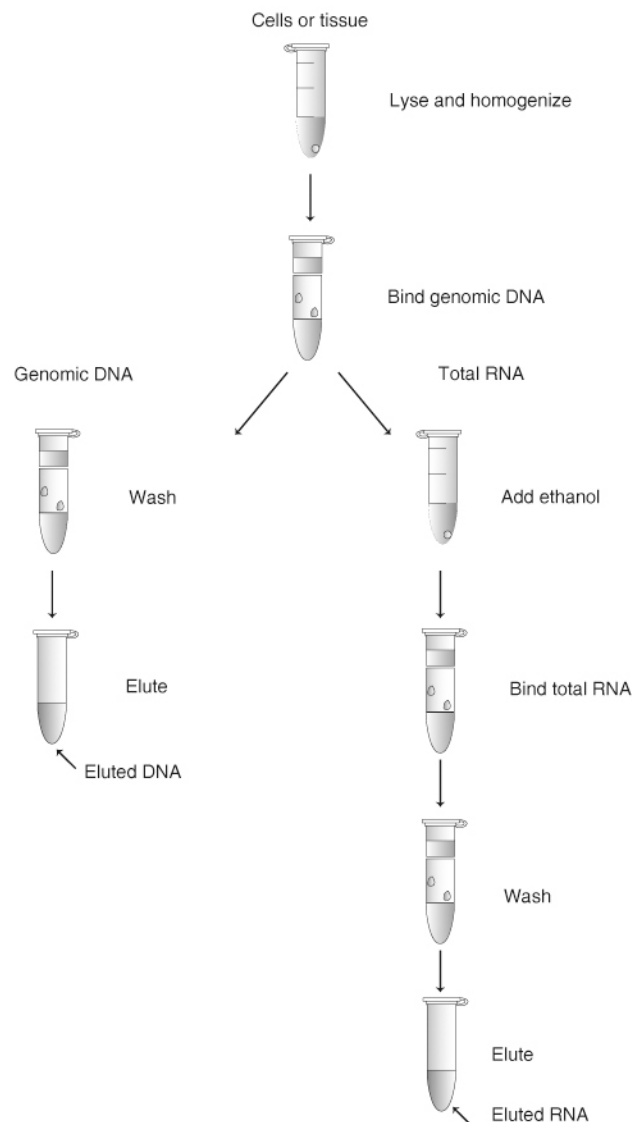
CE Buffer is 10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0. Water can be used as elution in the DNA finalsteps houle EDTA be avoided for the following applications. However, this is not recommended if the pH of water is less than 7.0.

CW1 Solution, CW2 Solution, GT Solution and NT Solution are supplied as concentrates. Before using for the first time, add **17 ml** ethanol to **13 ml** CW1 Solution, **21 ml** ethanol to **9 ml** CW2 Solution, **12 ml** ethanol to **18 ml** GT solution and **24 ml** ethanol to **6 ml** NT solution to make a working solution.

	Ethanol (96-100%)	Final Volume
CW1 Solution (13 ml)	17 ml	30 ml
CW2 Solution (9 ml)	21 ml	30 ml
GT solution (18 ml)	12 ml	30 ml
NT solution (6 ml)	24 ml	30 ml

**PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC
RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR A NIMAL USE!**

■ DNA/RNA Isolation Procedure



■ 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, since this leads to RNA degradate.

B. Animal tissue: Grind 15–30 mg animal tissue to fine powder in liquid nitrogen. Transfer the frozen powder to 1.5 ml RNase-free centrifuge tube, and allow the liquid nitrogen to evaporate.

- ✓ 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 adding Buffer Lysis-DR.

C. Plant: Grind 25–50 mg plant tissue to fine powder in liquid nitrogen. Transfer the frozen powder to 1.5 ml RNase-free centrifuge tube, and allow the liquid nitrogen to evaporate.

- ✓ Do not let the tissue sample thaw before adding Buffer Lysis-DR.

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

3. Centrifuge at $12,000 \times g$ for 3 minutes at 4°C . Transfer the supernatant to a new RNase-Free tube.

● Genomic DNA Purification

4. Place the EZ-10 DNA 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. Transfer the flow-through to a new 1.5 ml RNase-Free centrifuge tube for RNA purification.

- ✓ Store the flow-through at 4°C or isolation RNA (step 11-16) before DNA wash and elute steps.

5. Add 350 µl Buffer Lysis-DR to the EZ-10 DNA Column, keep at room temperature for 1 minute, centrifuge at 9,000 x g for 1 minute at room temperature, discard the liquid in the collection tube.
6. Place the EZ-10 DNA Column in the collection tube, and add 500 µl CW1 Solution to the EZ-10 DNA Column, keep at room temperature for 1 minute, centrifuge at 9,000 x g for 1 minute at room temperature, discard the liquid in the collection tube.

✓ Check the label to ensure CW1 Solution was diluted with ethanol.

7. Place the EZ-10 DNA Column in the collection tube, and add 500 µl CW2 Solution to the EZ-10 DNA Column, keep at room temperature for 1 minute, centrifuge at 9,000 x g for 1 minute at room temperature, discard the liquid in the collection tube.

✓ Check the label to ensure CW2 Solution was diluted with ethanol.

8. Place the EZ-10 DNA Column in the collection tube, and centrifuge at 9,000 x g for 2 minutes at room temperature.

9. Incubate the open spin column at room temperature for 2-3 minutes until the ethanol has completely evaporated. Transfer the spin column to a clean 1.5 ml centrifuge tube.

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

10. Add 50 µl CE Buffer directly onto the center part of EZ-10 DNA Column membrane. Incubate at room temperature for 2 minutes, and then centrifuge for 2 minutes at 9,000 x g to elute the DNA.

✓ Warm the Buffer CE to 60°C will increase the elution efficiency.
 ✓ Elution with more than 50 µl (e.g. 100 µl) increases the DNA yield, but the concentration will be lower.
 ✓ For maximum DNA yield, repeat elution once as described in this step.
 ✓ A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first elution.

● **Total RNA Purification**

NOTE: 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 times.

11. Add 250 µl ethanol to the flow-through from step 4, mix thoroughly.

12. Place the RZ-10 RNA Column in the collection tube and transfer mixture to the RZ-10 RNA Column, centrifuge at 9,000 x g for 1 minute at room temperature. Discard the flow-through.

13. Add 500 µl GT Solution to the column, 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.

14. Add 500 µl NT Solution to the column, keep at room temperature for 1 minutes, 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.

15. Place the column in the collection tube, and centrifuge at 9,000 x g for 2 minutes at room temperature.

✓ 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.

16. Transfer the column into a new RNase-Free centrifuge tube, 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.

■ General Guidelines

All-In-One DNA/RNA Mini-Preps Kit provides a new technology for selective binding of double-stranded DNA with EZ-10 DNA Column.

Biological samples are first lysed and homogenized in a highly denaturing guanidine-containing buffer (buffer lysis-DR), which immediately inactivates DNases and RNases to ensure isolation of intact DNA, RNA.

DNA in the whole homogenate is selectively absorbed on EZ-10 DNA Column. wash the membrane with the buffer lysis-DR to remove RNA/Protein contaminants on the EZ-10 DNA spin column. PCR inhibitors, protein and salts are completely removed with CW1 solution and CW2 solution. Purified genomic DNA is eluted in CE buffer.

Ethanol is added to the flow-through from the EZ-10 DNA spin column to provide appropriate binding conditions for RNA, and the sample is then applied to a RZ-10 spin column, where total RNA binds to the membrane. PCR inhibitors, protein and salts are completely removed with GT Solution and NT Solution. Purified RNA is eluted in RNase-Free water.

■ Notes for Sample Preparation

● Sample Collection and Storage

Best results are obtained with fresh samples. When purifying total DNA/RNA from fresh samples, keep fresh cell and tissue samples in liquid nitrogen or on ice immediately after harvesting. Quickly proceed to sample lysis and homogenization.

If the samples need to be stored before using they should be frozen in liquid nitrogen or in RNALater (code: RT4171), and at -80°C for long term storage immediately. Avoid repeated freezing and thawing of stored samples, since this leads to DNA/RNA degradation.

● Sample Homogenize

We recommend treating the tissue samples with liquid nitrogen and proceeding to sample lysis in Buffer Lysis-DR/Buffer Lysis-DRP immediately. However, homogenizer may also be used successfully.

Work quickly during sample harvesting and homogenizing, and always wear disposable gloves while handling samples and reagents to prevent RNase contamination.

Use precool mortar, pestle and spatula to treat and transfer the samples. Do not let the tissue sample thaw before you add the Lysis-DR/Buffer Lysis-DRP.

● Starting Amounts of Samples

The yield and quality of DNA/RNA depends on the amount of starting material. It is important not to exceed the capacity of lysis buffer and membrane. Use advisable amount of starting material as follows:

Sample	Amount
Muscle tissue	30 mg
Liver or brain tissue	20 mg
Kidney or spleen tissue	10 mg
Cultured cells	1 x 10 ⁷
Plant tissue	50 mg

■ Quantity and quality of genomic DNA

Quantity and quality of genomic DNA can be simply determined using two methods: absorbance (determine the concentration and purity of genomic DNA), agarose gel electrophoresis (determine the concentration and length of genomic DNA).

● UV Spectrophotometric Analysis of DNA

DNA strongly absorb ultraviolet light at 260 nm. UV spectroscopy can be used as a quantitative technique to measure DNA concentration and purity. For greatest accuracy, readings should be between 0.1 and 1.0. You should ensure the concentrations of DNA solution are higher than 2.5 ng/μl. You can calculate the concentration of the DNA in your sample as follows:

$$1 \text{ OD (optical density) at } A_{260} = 50 \text{ } \mu\text{g/ml dsDNA}$$

$$\text{DNA concentration } (\mu\text{g/ml}) = (\text{OD}_{260}) \times (\text{dilution factor}) \times (50 \text{ } \mu\text{g DNA/ml}) / (1 \text{ OD}_{260} \text{ unit})$$

$$\text{DNA yield } (\mu\text{g}) = \text{DNA concentration} \times \text{total DNA volume (ml)}$$

The reading at 280 nm determines the amount of protein in a sample. The $\text{OD}_{260}/\text{OD}_{280}$ is an indicator of DNA purity. You can estimate the purity of the DNA in your sample as follows:

$\text{OD}_{260}/\text{OD}_{280}$ of purified DNA is generally 1.7-1.9. The ratio can be calculated after correcting for turbidity (absorbance at 320 nm).

$$\text{DNA Purity } (\text{OD}_{260}/\text{OD}_{280}) = (\text{OD}_{260} \text{ reading} - \text{OD}_{320} \text{ reading}) \div (\text{OD}_{280} \text{ reading} - \text{OD}_{320} \text{ reading}).$$

If there is contamination with proteins or phenol, the $\text{OD}_{260}/\text{OD}_{280}$ will be less than 1.6, and accurate quantitation of the amount of nucleic acid will not be possible.

It is also helpful when looking at the purity of DNA to take an absorbance reading at 230 nm. Strong absorbance around 230 nm can indicate that organic compounds or chaotropic salts are present in the purified DNA. A ratio between the readings at 260 nm and 230 nm ($\text{OD}_{260}/\text{OD}_{230}$) can help to evaluate the level of salt carryover in the purified nucleic acid. Generally, the $\text{OD}_{260}/\text{OD}_{230}$ of DNA is greater than 1.8.

● Agarose gel electrophoresis

Agarose gel electrophoresis is a method used to estimate the size of DNA fragments or the concentration of DNA. Regular agarose gels may range in concentration from 0.7 to 1.0%.

DNA possesses a consistent charge to mass ratio, therefore the major factor influencing migration through a gel matrix is size (and occasionally secondary structure). Fragment sizes can be determined by comparison to standard DNA size markers.

Lambda DNA/*Hind*III marker (BSM0103) is recommend as standard DNA size marker. Lambda DNA/*Hind*III marker is premixed with DNA loading dye at a final DNA concentration of 0.1 μg/μl. It contains 8 discrete fragments (from 125 to 23130 bp). The reference size is 23130 base pairs in length. Apply 5 μl (0.5 μg) of the DNA marker on a 5 mm lane of agarose gel. Compare fragment (s) of unknown concentrations with Lambda DNA/*Hind*III marker by calculating DNA concentration of as follows: The band (23130 bp) that you will use as a reference is 47.7% of the total fragment(s). There is 239 ng 23130 bp in 0.5 μg Lambda DNA/*Hind*III marker. Analysis the gray scale value of sample electrophoresis bands and standard DNA size marker, you can estimate the concentration of sample genomic DNA by software.

■ Quantity and quality of total RNA

Quantity and quality of total RNA can be simply determined using two methods: absorbance (determine the concentration and purity of total RNA), formaldehyde agarose gel electrophoresis (determine the integrity of total RNA).

● UV Spectrophotometric Analysis of DNA

RNA is similar with DNA has an absorption peak at 260 nm.

You can calculate the concentration of the RNA in your sample as follows:

$$1 \text{ OD (optical density) at } A_{260} = 40 \text{ } \mu\text{g/ml ssRNA}$$

$$\text{RNA concentration } (\mu\text{g/ml}) = (\text{OD}_{260}) \times (\text{dilution factor}) \times (40 \text{ } \mu\text{g RNA/ml}) / (1 \text{ OD}_{260} \text{ unit})$$

$\text{OD}_{260}/\text{OD}_{280}$ of purified RNA is generally 1.9-2.1.

If there is contamination with genomic DNA or protein, the $\text{OD}_{260}/\text{OD}_{280}$ will be less than 1.8. If chaotropic salts are present in the purified RNA, the $\text{OD}_{260}/\text{OD}_{280}$ will be greater 2.1.

Generally, the $\text{OD}_{260}/\text{OD}_{230}$ of RNA is greater than 2.0. If the ration of $\text{OD}_{260}/\text{OD}_{230}$ is less than 2.0, the RNA solution may be contain chaotropic salts.

● Formaldehyde agarose gel electrophoresis

The integrity and size distribution of total RNA purified can be checked by denaturing-agarose gel electrophoresis and ethidium bromide staining. According to the size of ribosomal RNAs, you can determine the integrity of total RNA.

Intact total RNA run on a denaturing gel will have sharp 28S and 18S rRNA bands (eukaryotic samples). The 28S rRNA band should be approximately twice as intense as the 18S rRNA band.

FA gel preparation: To prepare FA gel (1.2% agarose) of size 10 x 14 x 0.7 cm, mix:

1.2 g agarose.

10 ml 10x FA gel buffer (see composition below)

Add RNase-free water to 100 ml

If smaller or larger gels are needed, adjust quantities of components proportionately. Heat the mixture to melt agarose. Cool to 65°C in a water bath. Add 1.8 ml of 37% (12.3 M) formaldehyde and 1 µl of a 10 mg/ml ethidium bromide stock solution. Mix thoroughly and pour onto gel support. Prior to running the gel, equilibrate in 1x FA gel running buffer (see composition below) for at least 30 minutes.

RNA sample preparation for FA gel electrophoresis

Add 1 volume of 5x RNA loading buffer (see composition below) to 4 volumes of RNA sample (for example, 10 µl of loading buffer and 40 µl of RNA) and mix. Incubate for 3–5 minutes at 65°C, chill on ice, and load onto the equilibrated FA gel.

Gel running conditions

Run gel at 5–7 V/cm in 1x FA gel running buffer.

Composition of FA gel buffers

10x FA gel buffer

200 mM	3-[N-morpholino]propanesulfonic acid (MOPS) (free acid)
50 mM	sodium acetate
10 mM	EDTA
pH to 7.0 with NaOH	

1x FA gel running buffer

100 ml	10x FA gel buffer
20 ml	37% (12.3 M) formaldehyde
880 ml	RNase-free water

5x RNA loading buffer

16 µl	saturated aqueous bromophenol blue solution
80 µl	500 mM EDTA, pH 8.0
720 µl	37% (12.3 M) formaldehyde
2 ml	100% glycerol
3.084 ml	formamide
4 ml	10x FA gel buffer
RNase-free water to 10 ml	
Stability: approximately 3 months at 4°C	

■ Troubleshooting Guide

1. DNA is contaminated with RNA

- The final homogenate should have a pH of 7.0. Make sure that the sample is not highly acidic or basic.
- Wash the EZ-10 DNA spin column once with Buffer Lysis-DR/Buffer Lysis-DRP to remove RNA contaminants.
- Add RNase to the DNA elution directly.

2. Low Yield of DNA

- Homogenize tissue completely. Treat the tissue samples with liquid nitrogen or homogenizer.
- Use advisable amount of starting material. DNA yield is dependent on the type, size, age and storage of starting material. Please increase the amount of starting of some plant tissue with low DNA content.
- Check the label to ensure CW1 Solution and CW2 Solution were diluted with ethanol respectively.
- Elution step has to be strictly followed. Please reference the note on page 9.
- Avoid overdrying the membrane of EZ-10 spin column. Incubate the membrane at room temperature 3-5 minutes to dry the membrane of the EZ-10 spin column. Do not leave the membrane at room temperature or 65°C for long-term.

3. RNA is contaminated with DNA

- Reduce the amount of starting material.
- For certain tissues with extremely high DNA content (e.g. thymus), some DNA will pass through the EZ-10 DNA spin column. Try using smaller sample size.
- Complete removal of cell-culture medium or stabilization reagent.

4. Low Yield of RNA

- We recommend using fresh samples.
- Homogenize tissue completely. Treat the tissue samples with liquid nitrogen or homogenizer.
- Use advisable amount of starting material. RNA yield is dependent on the type, size, age and storage of starting material. Please reference the direction on page 21.
- Check the label to ensure GT Solution and NT Solution were diluted with ethanol respectively.
- Elution step has to be strictly followed.

5. RNA degradation

- 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 in Buffer Lysis-DR/Buffer Lysis-DRP 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.

6. Clumping in binding steps

- A. Make sure samples are homogenized completely before binding the spin column.
- B. Centrifuge at $12,000 \times g$ for 3 minutes to remove the fibres and cell debris before binding the spin column.
- C. Reduce the amount of starting material. Use advisable amount of starting material as direction on page 21.
- D. Check with Buffer Lysis-DR/Buffer Lysis-DRP, if forms precipitates upon storage, warm to 56°C to dissolve it before use.

7. Inhibition of downstream reactions

- A. Residual ethanol from the CW2 Solution can inhibit downstream enzymatic reactions. Centrifuge the column at $12,000 \times g$ for 2 minutes and incubate the membrane at room temperature 3-5 minutes to dry the membrane of the EZ-10 spin column to remove the residual ethanol thoroughly.
- B. Residual salt can inhibit downstream enzymatic reactions. Ensure that wash steps have been operated at room temperature ($15\text{--}25^{\circ}\text{C}$).

8. Difficult to dissolve protein pellet

- A. Resuspend/disturb the pellet by pipetting up and down several times. Then briefly centrifuge the sample, and use the supernatant for downstream analysis.
- B. Several types of protein are very difficult to solubilize, especially membrane proteins. To improve solubility, use a different resuspension buffer containing other detergent(s) more suitable for your protein of interest.
- C. For greater solubilization of proteins, dissolve the protein pellet in 5% (w/v) SDS or 8 M urea, or increase the volume of PD Solution.

9. Protein shows no clear pattern in SDS-PAGE

- A. When washing the protein pellet with 50% ethanol, disturb the pellet by pipetting up and down several times.
- B. The quality of SDS-PAGE can be influenced by several parameters independent of protein quality. Vary the protein load and/or the polyacrylamide concentration of the gel (which should be according to molecular mass of the protein of interest). Incubation of the sample for 10 minutes at 46°C before loading (instead of 95°C) can improve the resolution.

■ Appendix

● The parameter of Column

	EZ-10 DNA Column	RZ-10 RNA Column
Colour	Blue	Colorless
Function	Binding DNA	Binding RNA
Maximum Binding Capacity	20 μg	20 μg
Maximum Loading Volume	750 μl	750 μl
Yes/No RNase-Free	Yes	Yes
Accepted Maximum Spin Speed	15,000 $\times g$	15,000 $\times g$

● Centrifugation Speed

Protocols for centrifugation typically specify the amount of acceleration to be applied to the sample, rather than specifying a rotational speed such as revolutions per minute. This distinction is important because two rotors with different diameters running at the same rotational speed will subject samples to different accelerations. This relationship between rpm and RCF ($\times g$) may be written as:

$$\text{rpm} = 1000 \times (\text{RCF}/1.12r)^{1/2}$$

r is the semidiameter of rotor
RCF means relative centrifugal force

● Precipitation of RNA with Ethanol.

1. **Add 0.1 volume of 3 M sodium acetate, pH 5.2 and 2.5 volumes of 96–100 % ethanol to one volume of sample. Mix thoroughly.**
2. **Incubate several minutes to several hours at -20°C or 4°C .**

✓ Note: Choose long incubation times if the sample contains low RNA concentration. Short incubation times are sufficient if the sample contains high RNA concentration.

3. **Centrifuge for 10 min at max. speed.**
4. **Wash RNA pellet with 70 % ethanol.**
5. **Dry RNA pellet and resuspend RNA in RNase-free H_2O .**



■ Ordering information

Code	Product Name	Size
BS88003	All-In-One DNA/RNA/Protein Mini-Preps Kit	50 Preps
BS88203	All-In-One DNA/RNA Mini-Preps Kit	50 Preps

■ Related Products

Code	Product Name	Size
BS410A	EZ-RNA Reagents	100 ml
BS583	EZ-10 Spin Column Bacterial Total RNA Mini-Preps Super 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
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
MT91928	mRNA Purification Kit from Total RNA	25 Preps