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

Introduction

All-In-One DNA/RNA/Protein Mini-preps Kit is designed for simultaneous extrac?on of total RNA, genomic DNA and protein from a single biological sample. DNA, RNA and protein are isolated without spli?ng the sample prior to extrac?on. DNA, RNA and Protein can be isolated from cultured eukaryo?c cells, animal and plant ?ssues.

This kit provides an innova?ve buffer system and a silica-based column nucleic acid purifica?on technology to separate pure genomic DNA from total RNA. There is no need for phenol/chloroform extrac?on. Purified DNA is suitable for downstream applica?ons such as Restric?on Endonuclease Diges?ons, PCR and other applica?ons. Isolated total RNA can be used for mRNA isola?on, probe genera?on, RT-PCR, Northern blot analysis, primer extension, RNA protec?on assay and In vitro transla?on.

Protein is purified in denatured form with a special buffer (PP Solu?on) which effec?vely precipitates protein. A?er washing steps, the protein pellet is dissolved in PD Solu?on. Isolated protein is suitable for SDS-PAGE, Western Blot analysis and quan?fica?on. The procedure is simple and fast. Genomic DNA, total RNA and Proteins can be isolated in less than 1 hour.

Features

- Genomic DNA, RNA and Proteins can be simultaneously isolated in less than 1 hour.
- Prepara?on of high quality genomic DNA with a molecular weight ≥20 kb.
- Prepara?on of high quality total RNA without genomic DNA.
- Isolated protein is suitable for SDS-PAGE and Western Blot analysis.
- No phenol/chloroform extrac?on or ethanol precipita?on is required.
- High yield and reproducibility.

All-In-One Mini-Preps Kit (Cell, Tissue, Plant)
BS88203

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Kit Contents

	BS88203, 50 Preps
Buffer Lysis-DRP	45 ml
CW1 Solu?on (concentrate)	13 ml
CW2 Solu?on (concentrate)	9 ml
CE Buffer	10 ml
GT Solu?on (concentrate)	18 ml
NT Solu?on (concentrate)	6 ml
RNase-Free Water	5 ml
PP Solu?on	35 ml
PD Solu?on	10 ml
EZ-10 DNA Column (blue) (with 2.0-ml Collec?on Tube)	50
RZ-10 RNA Column (uncolored) (with 2.0-ml Collec?on Tube)	50
Protocol	1

Storage Conditions

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

Applications

Direct correla?on between gene?c and proteomic data.

Efficient of up to 10 µg of genomic DNA purifica?on from animal ?ssue. Purified genomic DNA has an average length of 20-30 kb. DNA of this length is par?cularly suitable for PCR, where complete denatura?on of the template is important to achieve the highest amplifica?on efficiency. The purified DNA is ready to use in any downstream applica?on, including:

- PCR and real-?me PCR
- Southern, dot and slot blot analyses
- Compara?ve genome hybridiza?on (CGH)
- Genotyping, SNP analysis

Efficient of up to 20 µg of total RNA purifica?on from animal ?ssue. The purified RNA is ready to use in any downstream applica?ons, including:

- RT-PCR
- Quan?ta?ve, real-?me RT-PCR
- Differen?al display
- cDNA synthesis
- Northern, dot and slot blot analyses
- Primer extension
- Poly A + RNA selec?on
- RNase/S1 nuclease protec?on

- Microarrays

Isola?on protein in denatured form. The purified protein is suitable for downstream applica?ons such as:

- 1D gel electrophoresis
- Western blo?ng

Quality Control

Each lot of All-In-One DNA/RNA/Protein Mini-preps Kit is tested against predetermined specifica?ons to ensure consistent product quality.

Materials Supplied by User

- Microcentrifuge capable of at least 12,000 × g
- RNase-Free pipe?es and pipe?e ?ps
- Vortexer
- Isopropanol
- RNase-Free ethanol (96-100%)
- 50% ethanol
- RNase-Free microcentrifuge tubes (1.5 ml)

Before Starting

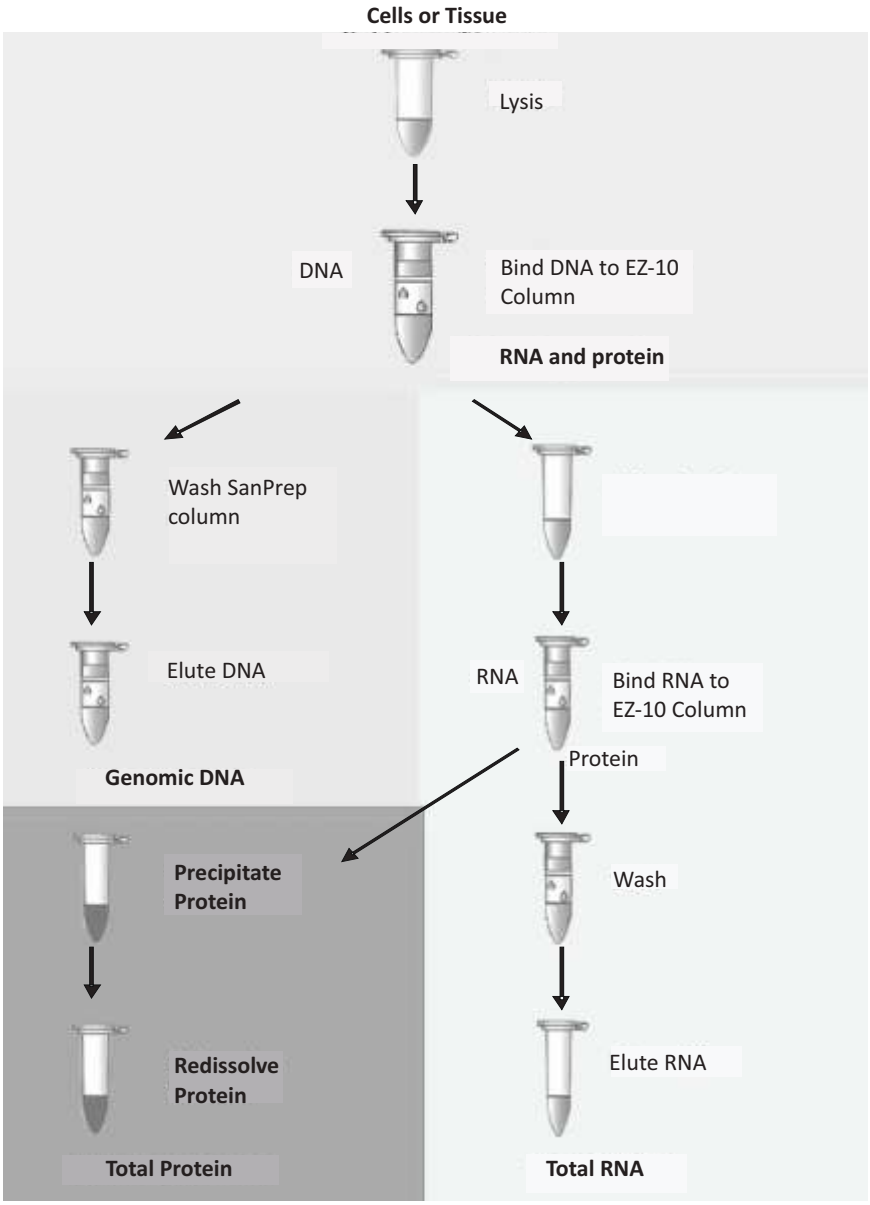
Check the Buffer Lysis-DRP for salt precipita?on before each use. If necessary, redissolve the precipitate by warming the solu?on at 56°C, then cool back down to room temperature before use.

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 applica?ons. However, this is not recommended if the pH of water is less than 7.0.

CW1 Solu?on, CW2 Solu?on, GT Solu?on and NT Solu?on are supplied as concentrates. Before using for the first ?me, add **17 ml** ethanol to **13 ml** CW1 Solu?on, **21 m**l ethanol to **9 ml** CW2 Solu?on, **12 ml** ethanol to **18 ml** GT Solu?on and **24 ml** ethanol to **6 ml** NT Solu?on to make a work solu?on.

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

DNA/RNA/Protein Isolation Procedure



Protocol

1. Sample prepara?on
   - **A.** Cell Cultures
     - **1a.** Cells grown in suspension: Spin appropriate number of cells (max. 1 x 10⁷) 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-DRP to the cell-culture dish. Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube. Vortex or pipe?e 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 extrac?on, it is recommended to store at -80°C for long-term.
       - Avoid repeated freezing and thawing of stored samples, since this leads to degradate RNA.
   - **B.** Animal ?ssue: Grind 15~30 mg animal ?ssue 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 ?ssues with a very high number of cells, such as spleen, no more than 10 mg star?ng material should be used.
     - Do not let the ?ssue sample thaw before adding Buffer Lysis-DRP.
   - **C.** Plant: Grind 25~50 mg plant ?ssue 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 ?ssue sample thaw before adding Buffer Lysis-DRP.
2. Add 350 µl 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 x 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 collec?on 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. Transfer the flow-through to a new RNase-Free tube for RNA purifica?on.
   - Store the flow-through at 4°C or isola?on RNA (step 11-17) before DNA wash and elu?on.
5. Add 350 µl Buffer Lysis-DRP 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 collec?on tube.
6. Place the EZ-10 DNA Column in the collec?on tube, and add 500 µl CW1 Solu?on 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 collec?on tube.
   - Check the label to ensure CW1 Solu?on was diluted with ethanol.

7. Place the EZ-10 DNA Column in the collec?on tube, and add 500 µl CW2 Solu?on 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 collec?on tube.
   - Check the label to ensure CW2 Solu?on was diluted with ethanol.
8. Place the EZ-10 DNA Column in the collec?on 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 un?l the ethanol has completely evaporated. Transfer the 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 reac?ons. This step ensures that no residual ethanol will be carried over during the following elu?on.
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 elu?on efficiency.
    - Elu?on with more than 50 µl (e.g. 100 µl) increases the DNA yield, but the concentra?on will be lower.
    - For maximum DNA yield, repeat elu?on once as described in this step.
    - A new microcentrifuge tube can be used for the second elu?on step to prevent dilu?on 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 star?ng with RNA sample prepara?on and con?nue through purifica?on and analysis. Use RNAse free tubes, ?ps, gels. Wear gloves at all ?mes.

11. Add 250 µl ethanol to the flow-through from step 4, mix thoroughly.
12. Place the RZ-10 RNA Column in the collec?on tube and transfer the 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 purifica?on.
14. Place the RZ-10 RNA Column in the collec?on tube, add 500 µl GT Solu?on, 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 Solu?on was diluted with ethanol.
15. Add 500 µl NT Solu?on 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 Solu?on was diluted with ethanol.
16. Place the column in the collec?on 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 reac?ons. This centrifuga?on step ensures that no residual ethanol will be carried over during the following elu?on.

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 solu?on in the centrifuge tube is the RNA sample, it can be used immediately for downstream molecular opera?on or stored at -70°C.

Total Protein precipitation

18. Add 600 µl PP Solu?on 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 pipe?ng up and down several ?mes.
21. Dry the protein pellet for 5-10 minutes at room temperature.
22. Add up to 100 µl PD Solu?on and mix thoroughly to dissolve the protein pellet. Protein dissolved in 5% (w/v) SDS can be quan?fied using the BCA method, but there must be no dye in the solu?ons.
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 solu?on in the centrifuge tube is the protein solu?on, it can be used immediately for downstream applica?ons such as SDS-PAGE and western blo?ng or stored at -20°C.

General Guidelines

All-In-One DNA/RNA/Protein Mini-preps Kit provides a new technology for selec?ve 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 precipita?on chemistry.

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

DNA in the whole homogenate is selec?vely absorbed on EZ-10 DNA Column. Wash the membrane with the buffer lysis-DRP to remove RNA/Protein contaminates on the EZ-10 DNA spin column. PCR inhibitors, protein and salts are completely removed with CW1 Solu?on and CW2 Solu?on. 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 condi?ons 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 Solu?on and NT Solu?on. Purified RNA is eluted in RNase-Free water.

Buffer PP Solu?on, a novel aqueous protein precipita?on solu?on, is added to the flow-through of the RZ-10 spin column, and the precipitated proteins are pelleted by centrifuga?on. Intact total proteins are redissolved in PD Solu?on and then ready to use in downstream applica?ons.

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

Introduction

All-In-One DNA/RNA Mini-preps Kit is designed for simultaneous extrac?on of total RNA and genomic DNA from a single biological sample. DNA and RNA are isolated without spli?ng the sample prior to extrac?on. DNA and RNA can be isolated from cultured eukaryo?c cells, animal and plant ?ssues.

This kit, using an innova?ve buffer system, requires no need for phenol/chloroform extrac?ons and provides column to separately pure genomic DNA and total RNA. Purified DNA is suitable for downstream applica?ons such as Restric?on Endonuclease Diges?ons, PCR, and so on. The isolated RNA can be used for mRNA isola?on, probe genera?on, RT-PCR, Northern blot analysis, primer extension, RNA protec?on assay and In vitro transla?on. The procedure is simple and fast. Genomic DNA and RNA can be isolated in less than 40 minutes.

Features

- Genomic DNA and total RNA can be simultaneously isolated in less than 40 minutes.
- Prepara?on of high quality genomic DNA with a molecular weight ≥20 kb.
- Prepara?on of high quality total RNA without genomic DNA.
- High yield and reproducible.
- No phenol/chloroform extrac?on or ethanol precipita?on is required.

Kit Contents

Component	BS88203, 50 Preps
Buffer Lysis-DR	45 ml
CW1 Solu?on (concentrate)	13 ml
CW2 Solu?on (concentrate)	9 ml
CE Buffer	10 ml
GT Solu?on (concentrate)	18 ml
NT Solu?on (concentrate)	6 ml
RNase-Free Water	5 ml
EZ-10 DNA Column (blue) (with 2.0-ml Collec?on Tube)	50
RZ-10 RNA Column (uncolored) (with 2.0-ml Collec?on Tube)	50
Protocol	1

Storage Conditions

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

All-In-One Mini-Preps Kit (Cell, Tissue, Plant)
BS88203

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Applications

Efficient of up to 10 µg of genomic DNA purifica?on from animal ?ssue. Purified genomic DNA has an average length of 20-30 kb. DNA of this length is par?cularly suitable for PCR, where complete denatura?on of the template is important to achieve the highest amplifica?on efficiency. The purified DNA is ready to use in any downstream applica?ons, including:

- PCR and real-?me PCR
- Southern, dot and slot blot analyses
- Compara?ve genome hybridiza?on (CGH)
- Genotyping, SNP analysis

Efficient of up to 20 µg of total RNA purifica?on from animal ?ssue. The purified RNA is ready to use in any downstream applica?on, including:

- RT-PCR
- Quan?ta?ve, real-?me RT-PCR
- Differen?al display
- cDNA synthesis
- Northern, dot and slot blot analyses
- Primer extension
- Poly A + RNA selec?on
- RNase/S1 nuclease protec?on
- Microarrays

Quality Control

Each lot of All-In-One DNA/RNA Mini-preps Kit is tested against predetermined specifica?ons to ensure consistent product quality.

Materials Supplied by User

- Microcentrifuge capable of at least 12,000 × g
- RNase-Free pipe?es and pipe?e ?ps
- Vortexer
- RNase-Free ethanol (96-100%)
- RNase-Free microcentrifuge tubes (1.5 ml)

Before Starting

Check the Buffer Lysis-DR for salt precipita?on before each use. If necessary, redissolve the precipitate by warming the solu?on 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 if EDTA should be avoided for the following applica?ons, but it is not recommended if the pH of water is less than 7.0.

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

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

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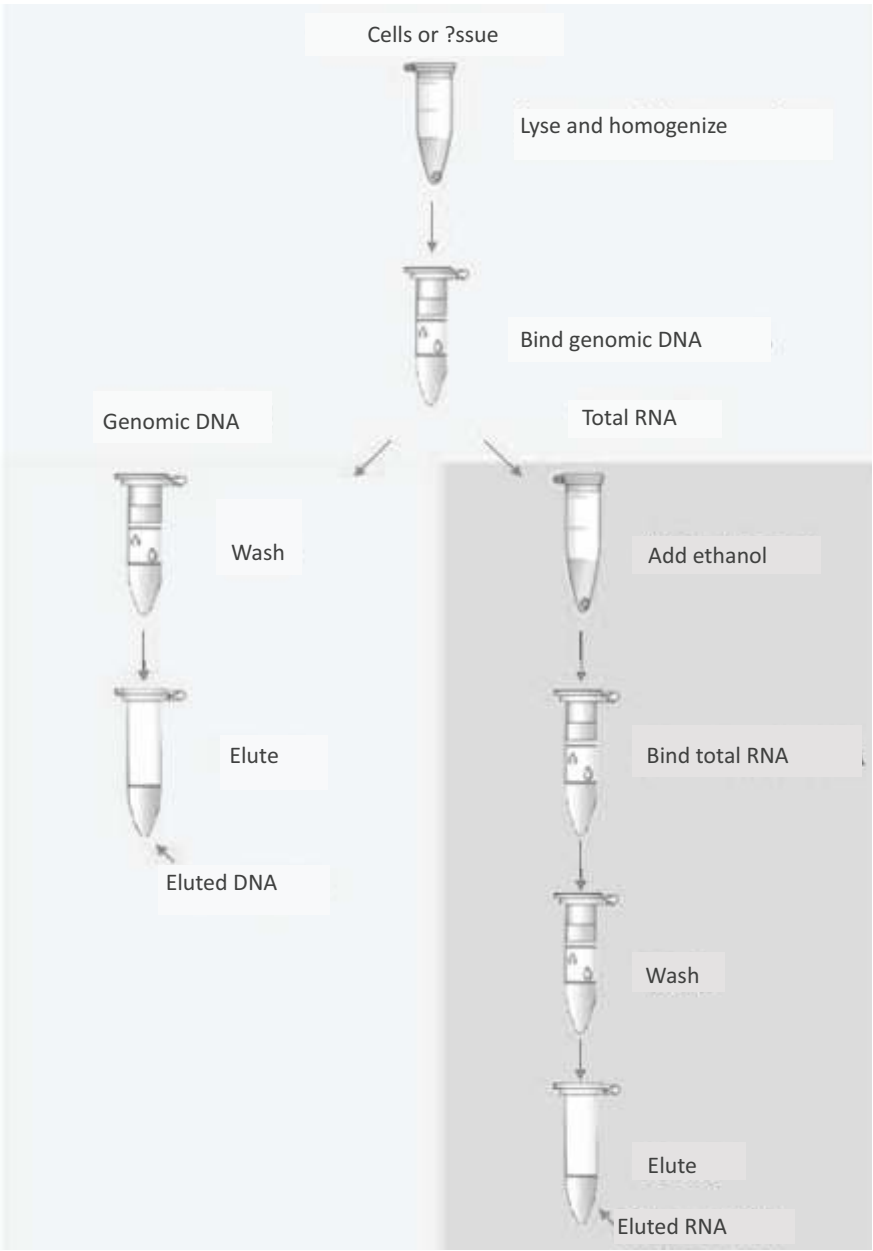
DNA/RNA Isolation Procedure



Protocol

1. Sample prepara?on
   - **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. Pipe?e the lysate into a microcentrifuge tube. Vortex or pipe?e 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 extrac?on, it is recommended to store at -80°C for long-term.
       - Avoid repeated freezing and thawing of stored samples, since this leads to RNA degrada?on.
   - **B.** Animal ?ssue: Grind 15~30 mg animal ?ssue 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 ?ssues with a very high number of cells, such as spleen, no more than 10 mg star?ng material should be used.
     - Do not let the ?ssue sample thaw before adding Buffer Lysis-DR.
   - **C.** Plant: Grind 25~50 mg plant ?ssue 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 ?ssue sample thaw before adding Buffer Lysis-DR.
2. Add 350 µl Buffer Lysis-DR immediately to the 1.5 ml RNase-Free centrifuge tube above, mix by vortex.
3. Centrifuge at 12,000 x 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 collec?on 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. Transfer the flow-through to a new RNase-Free tube for RNA purifica?on.
   - Store the flow-through at 4°C or isola?on RNA (step 11-16) before DNA wash and elu?on.
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 collec?on tube.
6. Place the EZ-10 DNA Column in the collec?on tube, and add 500 µl CW1 Solu?on 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 collec?on tube
   - Check the label to ensure CW1 Solu?on was diluted with ethanol.
7. Place the EZ-10 DNA Column in the collec?on tube, and add 500 µl CW2 Solu?on 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 collec?on tube.

- Check the label to ensure CW2 Solu?on was diluted with ethanol.

8. Place the EZ-10 DNA Column in the collec?on 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 un?l the ethanol has completely evaporated. Transfer the 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 reac?ons. This step ensures that no residual ethanol will be carried over during the following elu?on.
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 elu?on efficiency.
    - Elu?on with more than 50 µl (e.g. 100 µl) increases the DNA yield, but the concentra?on will be lower.
    - For maximum DNA yield, repeat elu?on once as described in this step.
    - A new microcentrifuge tube can be used for the second elu?on step to prevent dilu?on 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 star?ng with RNA sample prepara?on and con?nue through purifica?on and analysis. Use RNAse free tubes, ?ps, gels. Wear gloves at all ?mes.

11. Add 250 µl ethanol to the flow-through from step 4, mix throughly.
12. Place the RZ-10 RNA Column in the collec?on tube and transfer the 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 Solu?on 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 Solu?on was diluted with ethanol.
14. Add 500 µl NT Solu?on 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 Solu?on was diluted with ethanol.
15. Place the column in the collec?on 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 reac?ons. This centrifuga?on step ensures that no residual ethanol will be carried over during the following elu?on.
16. Transfer the column into a new RNase-Free centrifue tube, add 30-50ul RNase-Free water, keep at room temperature for 2 minutes, and centrifuge at 9,000 x g for 2 minutes.
    - The solu?on in the centrifuge tube is the RNA sample. It can be used immediately for downstream molecular opera?on or stored at -70°C.

Quantity and quality of total RNA

Quan?ty and quality of total RNA can be simply determined using two methods: absorbance (determine the concentra?on 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 a absorp?on peak at 260 nm.
You can calculate the concentra?on of the RNA in your sample as follows:

- 1 OD (op?cal density) at A_{260} = 40 µg/ml ssRNA
- RNA concentra?on (µg/ml) = (OD_{260}) x (dilu?on factor) x (40 µg RNA/ml)/(1 OD_{260} unit)
- $OD_{260}/_{280}$ of purified RNA is generally 1.9-2.1.
- If there is contamina?on with genomic DNA or protein, the OD_{260}/OD_{280} will be less than 1.8. If chaotropic salts are present in the purified RNA, the OD_{260}/OD_{280} will be greater than 2.1.
- Generally, the OD_{260}/OD_{230} of RNA is greater than 2.0. If the ra?on of OD_{260}/OD_{230} is less than 2.0, the RNA solu?on may be contain chaotropic salts.

Formaldehyde agarose gel electrophoresis

The intergrity and size distribu?on 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 (eukaryo?c 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 composi?on below)
- Add RNase-free water to 100 ml

If smaller or larger gels are needed, adjust quan??es of components propor?onately. 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 solu?on. Mix thoroughly and pour onto gel support. Prior to running the gel, equilibrate in 1x FA gel running buffer (see composi?on below) for at least 30 minutes.

RNA sample preparation for FA gel electrophoresis

Add 1 volume of 5x RNA loading buffer (see composi?on 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 solu?on
- 80 µl 500 mM EDTA, pH 8.0
- 720 µl 37% (12.3 M) formaldehyde
- 2ml 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

General Guidelines

All-In-One DNA/RNA Mini-preps Kit provides a new technology for selec?ve binding of double-stranded DNA with EZ-10 DNA Column, and binding of total RNA with RZ-10 RNA Column.

Biological samples are first lysed and homogenized in a highly denaturing guanidine-containing buffer (buffer lysis-DR), which immediately inac?vates DNases and RNases as well as proteases to ensure isola?on of intact DNA and RNA.

DNA in the whole homogenate is selec?vely absorbed on EZ-10 DNA Column. Wash the membrane with the buffer lysis-DR to remove RNA/Protein contaminates on the EZ-10 DNA spin column. PCR inhibitors, protein and salts are completely removed with CW1 Solu?on and CW2 Solu?on. 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 condi?ons 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 Solu?on and NT Solu?on. 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 ?ssue samples in liquid nitrogen or on ice immediately a?er harves?ng. Quickly proceed to sample lysis and homogeniza?on.

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

Sample Homogenize

We recommend trea?ng the ?ssue samples with liquid nitrogen and proceeding to sample lysis immediately. However, homogenizer may also be used successfully.

Work quickly during sample harves?ng and homogenizing, and always wear disposable gloves while handling samples and reagents to prevent RNase contamina?on.

Use pre-cool mortar, pestle and spatula to treat and transfer the samples. Do not let the ?ssue sample thaw before you add the Lysis-DR/Buffer Lysis-DR.

Starting amounts of samples

The yield and quality of DNA/RNA depend on the amount of star?ng material. It is important not to exceed the capacity of lysis buffer and membrane. Use advisable amount of star?ng material as follow:

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

Quantity and quality of genomic DNA

Quan?ty and quality of genomic DNA can be simply determined using two methods: absorbance (determine the concentra?on and purity of genomic DNA), agarose gel electrophoresis (determine the concentra?on 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 quan?ta?ve technique to measure DNA concentra?on and purity. For greatest accuracy, readings should be between 0.1 and 1.0. You should be ensure the concentra?ons of DNA solu?on are higher than 2.5 ng/µl.You can calculate the concentra?on of the DNA in your sample as follows:

- 1 OD (op?cal density) at A_{260} = 50 µg/ml dsDNA
- DNA concentra?on (µg/ml) = (OD_{260}) x (dilu?on factor) x (50 µg DNA/ml)/(1 OD_{260} unit)
- DNA yield (µg) = DNA concentra?on × total DNA volume (ml)

The reading at 280 nm determines the amount of protein in a sample. A ra?o of the OD_{260}/OD_{280} is an indicator of DNA purity. You can es?mate the purity of the DNA in your sample as follows:

- $OD_{260}/_{280}$ of purified DNA is generally 1.7-1.9. The ra?o can be calculated a?er correc?ng for turbidity (absorbance at 320 nm).
- DNA Purity (OD_{260}/OD_{280}) = (OD_{260} reading – OD_{320} reading) ÷ (OD_{280} reading - OD_{320} reading).

If there is contamina?on with proteins or phenol, the OD_{260}/OD_{280} will be less than 1.6, and accurate quan?ta?on 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 ra?o between the readings at 260 nm and 230 nm (OD_{260}/OD_{230}) can help to evaluate the level of salt carryover in the purified nucleic acid. Generally, the OD_{260}/OD_{230} of DNA is greater than 1.8.

Agarose gel electrophoresis

Agarose gel electrophoresis is a method used to es?mate the size of DNA fragments or the concentra?on of DNA. Regular agarose gels may range in concentra?on from 0.7 to 1.0%

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

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

Troubleshooting

DNA is contaminated with RNA

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

Low Yield of DNA

A. Homogenize ?ssue completely. Treat the ?ssue samples with liquid nitrogen or homogenizer.
B. Use advisable amount of star?ng material. DNA yield is dependent on the type, size, age and storage of star?ng material. Please increase the mount of star?ng of some plant ?ssue with low DNA content.
C. Check the label to ensure CW1 Solu?on and CW2 Solu?on were diluted with ethanol respec?vely.
D. Elu?on step has to be strictly followed. Please reference the note on page 7.
E. 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.

RNA is contaminated with DNA

A. Reduce the amount of star?ng material.
B. For certain ?ssues with extremely high DNA content (e.g., thymus), some DNA will pass through the EZ-10 DNA spin column. Try using smaller sample size.
C. Complete removal of cell-culture medium or stabiliza?on reagent.

Low Yield of RNA

A. We recommend using fresh samples.
B. Homogenize ?ssue completely. Treat the ?ssue samples with liquid nitrogen or homogenizer.
C. Use advisable amount of star?ng material. RNA yield is dependent on the type, size, age and storage of star?ng material. Please reference the direc?on on page 17.
D. Check the label to ensure GT Solu?on and NT Solu?on were diluted with ethanol respec?vely.
E. Elu?on step has to be strictly followed.

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 trea?ng the ?ssue samples with liquid nitrogen and proceeding to sample lysis in Buffer Lysis-DRP or Buffer Lysis-DR immediately.
C. Create a RNase-Free working environment.
D. Wear gloves during all steps of the procedure. Change gloves frequently.
E. Using of RNase-Free microcentrifuge tubes is recommended.

Clumping in binding steps

A. Make sure samples are homogenized completely before binding the spin column.
B. Centrifuge at 12,000 x g for 3 minutes to remove the fibres and cell debris before binding the spin column.
C. Reduce the amount of star?ng material. Use advisable amount of star?ng material as direc?on on page 17.
D. Check Buffer Lysis-DRP or Buffer Lysis-DR, if form precipitates upon storage, warm to 56°C to dissolve it before use.

Inhibition of downstream reactions

A. Residual ethanol from the CW2 Solu?on can inhibit downstream enzyma?c reac?ons. Centrifuge the column at 12,000 × 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 throughly.
B. Residual salt can inhibit downstream enzyma?c reac?ons. Ensure that wash steps have been operated at room temperature (15–25°C).

Difficult to dissolve protein pellets

A. Resuspend/disturb the pellet by pipe?ng up and down several ?mes. 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 solubiliza?on of proteins, dissolve the protein pellet in 5% (w/v) SDS or 8 M urea, or increase the volume of PD Solu?on.

Protein shows no clear pattern in SDS-PAGE

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

Appendix

The parameter of Column

	EZ-10 DNA Column	RZ-10 RNA Column
Colour	Blue	Colorless
Func?on	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 x g	15,000 x g

Centrifugation Speed

Protocols for centrifuga?on typically specify the amount of accelera?on to be applied to the sample, rather than specifying a rota?onal speed such as revolu?ons per minute. This dis?nc?on is important because two rotors with different diameters running at the same rota?onal speed will subject samples to different accelera?ons. This rela?onship between rpm and RCF (x g) may be wri?en as:

rpm = 1000 × (RCF/1.12r)½
r is the semidiameter of rotor
RCF means rela?ve 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 incuba?on ?mes if the sample contains low RNA concentra?on. Short incuba?on ?mes are sufficient if the sample contains high RNA concentra?on.
3. Centrifuge for 10 minutes at maximum speed.
4. Wash RNA pellet with 70 % ethanol.
5. Dry RNA pellet and resuspend RNA in RNase-free $_2$HO.

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Ordering Information

All-In-One Mini-Preps Kit

Code	Product Name	Size
BS88203	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	100ml
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 Extrac?on Kit	50 Preps
BS82313	EZ-10 Spin Column Blood RNA Mini-Preps Kit	50 Preps
BS88583	EZ-10 Total RNA Mini-prep Kit	50 Preps
BS88583	EZ-10 Total RNA Mini-prep Kit	50 Preps
BS88584	EZ-10 Total RNA Mini-prep Kit	100 Preps
BS88586	EZ-10 Total RNA Mini-prep Kit	250 Preps
BS88254	RNase-Free DNase Set	50 Preps
BS88133	EZ-10 DNAaway RNA Mini-prep Kit	50 Preps
BS88133	EZ-10 DNAaway RNA Mini-prep Kit	250 Preps
MT91928	mRNA Purifica?on Kit from Total RNA	25 Preps