

**MB583**

**HiPurA® DNA/ RNA Purification Kit**

**Kit Contents**

Product Code	Reagents provided	MB583		
		20 Preps	50 Preps	250 Preps
DS0090	Resuspension Buffer (MS)	5.6 ml	14 ml	70 ml
DS0010	Lysis Solution (C1)	6 ml	15 ml	75 ml
DS0014	Gram- Positive Lysis Solution (GPLA)	6 ml	15 ml	75 ml
DS0011	Prewash Solution Concentrate (PW)	6 ml	15 ml	75 ml
DS0012	Wash Solution Concentrate (WS)	8 ml	20 ml	100 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	6 ml	15 ml	75 ml
MB086	Proteinase K	10 mg	25 mg	125 mg
MB098	Lysozyme	0.27 g	0.675 g	3.375 gm
DS0003	RNase A Solution (20 mg/ml)	0.5 ml	1.25 ml	6.25 ml
DS0037	RNA Lysis Solution (HRL)	16 ml	40 ml	200 ml
DS0041	Prewash Solution (RW1)	18 ml	45 ml	225 ml
DS0042	Elution Solution (RNase- Free Water)	2.4 ml	6 ml	30 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos	250 nos
DSCA02	HiShredder [in DBCA016 Collection Tube]	20 nos	50 nos	250 nos
DBCA016	Collection Tubes (Uncapped), Polypropylene(2.0 ml)	20 nos	50 nos	250 nos
PW1139	Collection Tubes, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

**Intended Use**

Recommended for isolation of DNA/ RNA from human/ animal blood, cells, tissue, gram positive and gram negative bacterial samples.

**Introduction**

HiPurA® DNA/ RNA Purification Kit provides a fast and easy method for purification of total DNA/ RNA from a wide variety of samples for downstream applications such as PCR, Southern blotting technique etc. The DNA/ RNA purification procedure using the miniprep spin column comprises of three steps viz. adsorption of DNA/ RNA to the membrane, removal of residual contaminants and elution of pure genomic DNA/ RNA. HiMedia's HiElute Miniprep Spin Column (Capped) format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality genomic DNA/ RNA is obtained from various species. The DNA/ RNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

### **HiPurA® DNA/ RNA Purification Kit**

This kit simplifies isolation of DNA/ RNA from fresh, old (more than 24 hours) and frozen blood, bacteria (Gram-positive and Gram-negative), tissues and cells with spin-column procedure.

Genomic DNA purification from blood involves cell lysis by incubating the whole blood in a solution containing chaotropic ions in the presence of Proteinase K at 55°C. After the initial binding of DNA onto the HiElute Miniprep Spin Column (Capped), impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality DNA is finally eluted in the Elution Buffer provided with the kit. Typical yield is 4-12 µg of total DNA from 200 µl of whole blood.

For genomic DNA isolation from bacteria, cells are grown in a medium till they reach log phase and are harvested by centrifugation. After harvesting, the bacterial (Gram-positive) cell wall is degraded by lysozyme and Proteinase K. For Gram-negative bacteria, the lysozyme treatment is not required. Following lysis, the DNA is bound to the silica-gel membrane of the HiElute Miniprep Spin Column (Capped) to yield approximately upto 20 µg of pure DNA. Two rapid wash steps remove trace amount of salt and protein contaminants resulting in the elution of pure DNA in the Elution Buffer provided with the kit.

Genomic DNA purification from animal tissue (spliced and digested), suspended/attached animal cells, digested rodent tail tissue is done by subjecting the samples to lysis by Proteinase K in a chaotropic salt solution. Following lysis is the binding of DNA to the silica gel membrane of the HiElute Miniprep Spin Column (Capped) to yield purified DNA. Wash steps remove trace salt and protein contaminants resulting in the elution of high quality DNA in the Elution Buffer (ET) provided with the kit. Typical DNA yield from  $1 \times 10^6$  cells is 10-20 µg and 30 mg of tissue yields 30-45 µg of DNA.

This kit simplifies isolation of RNA from animal cells, animal tissues and for cleanup of RNA from crude enzymatic reactions (e.g. DNase digestion, Proteinase digestion, RNA ligation, and labeling reaction) with spin-column procedure. The lysis buffer provided in the kit helps in cell disruption and denaturation, samples are centrifuged through a HiShredder which removes insoluble material and reduces the viscosity of the lysate by disrupting viscous material. Ethanol is added to the cleared lysate, which promotes selective binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane. After the initial binding of RNA, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality RNA is finally eluted in the Elution Solution provided with the kit. A specialized high-salt buffer system allows upto 100 µg of total RNA longer than 200 bases to bind to the HiElute Miniprep Spin column (Capped) membrane.

### **HiElute Miniprep Spin Column (Capped) [DBCA03]**

HiElute Miniprep Spin Column (Capped) is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced gel membrane and the speed plus versatility of spin column technology to yield high quantity of DNA/ RNA. The use of spin column facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. This column eliminates the need for alcohol precipitation, expensive resins and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional DNA/ RNA isolation techniques. DNA/ RNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer provided with the kit.

## Elution

The yield of genomic DNA/RNA depends on the sample type and the number of cells in the sample. Elution with 30-200 µl of Elution Buffer (ET) will provide sufficient DNA/RNA to carry out multiple amplification reactions. Elution with volume less than 200 µl will increase the final DNA/RNA concentration, but will reduce the overall DNA/RNA yield.

## Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer (ET) to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. The  $A_{260} - A_{320} / A_{280} - A_{320}$  ratio should be 1.6-1.9. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. DNA purified by HiPurA® DNA/RNA Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of DNA sample (µg/ml) =  $50 \times A_{260} \times$  dilution factor.

## Concentration, yield and purity of RNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the RNA. Use Elution Solution to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260nm, 280nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260nm should fall between 0.1 and 1.0. The 320nm absorbance is used to correct background absorbance. An absorbance of 1.0 at 260nm corresponds to approximately 40 µg/ml of RNA. The  $A_{260} - A_{320} / A_{280} - A_{320}$  ratio should be 1.8-2.1. Purity is determined by calculating the ratio of absorbance at 260nm to absorbance at 280nm. RNA purified by HiPurA® DNA/ RNA Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of RNA sample (µg/ml) =  $40 \times A_{260} \times$  dilution factor

## Materials needed but not provided

- 55°C water bath or heating block (For bacteria, tissues, rodent tails, blood)
- 37°C water bath or heating block (For Gram-positive bacteria only)
- 70°C water bath or shaking water bath (For cultured cells and tissues)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 - 100%)
- Molecular Biology Grade Water (Product code: ML024)
- Trypsin (Product code: TCL034)
- RNase – free pipette tips (aerosol barrier recommended)
- 2- mercaptoethanol (β -ME) (Product Code: MB041)
- Deoxyribonuclease I Solution (RNase-Free) and DNase Digest Buffer (procure from any standard company)
- TE Buffer (10 mM Tris Cl, 1mM EDTA pH 8.0) (Product Code: ML012/ML060)
- Ethanol (70%)

## Storage

Store the HiPurA® DNA/ RNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 1 year.

### General Preparation Instructions

1. Preheat a water bath or heating block to 55°C.  
(For Gram-positive, Gram-negative bacteria, blood, tissues and rodent tail)
2. Preheat a water bath or heating block to 37°C.  
(For Gram-positive bacteria only)
3. Preheat a water bath or heating block to 70°C.  
(For cultured cells and tissues)
4. **Thoroughly mix reagents**  
Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves. The reagent should be at room temperature (15-25°C) before use.
5. Ensure that clean & dry tubes and tips are used for the procedure.

6. **Dilute Prewash Solution Concentrate (PW) (DS0011) as follows:**

Number of Preps	Prewash Solution Concentrate (PW)	Ethanol (96-100%)
20	6 ml	9 ml
50	15 ml	22.5 ml
250	75 ml	112.5 ml

7. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
20	8 ml	24 ml
50	20 ml	60 ml
250	100 ml	300 ml

8. **Reconstitute Proteinase K (MB086)**

The HiPurA® DNA/ RNA Purification Kit contains Proteinase K. Intensive research has shown that it is the optimal enzyme for use with the Lysis Solution provided in the kit. It is completely free of DNase and RNase activity. Proteinase K is the enzyme of choice for use with an SDS containing Lysis Solution. The specific activity of Proteinase K is 33.5 units/mg dry weights.

Resuspend the Proteinase K (MB086) powder in Molecular Biology Grade Water (ML024) to obtain a 20 mg/ml stock solution.

Number of Preps	Proteinase K	Molecular Biology Grade Water
20	10 mg	0.5 ml
50	25 mg	1.25 ml
250	125 mg	6.25 ml

The product as supplied is stable at room temperature, upon reconstitution store at -20°C as mentioned in storage instructions.

**NOTE:** The Proteinase K solution must be added directly to each sample preparation every time. Do not combine the Proteinase K and Lysis solutions for storage.

9. **Prepare Lysozyme Solution (For Gram-positive bacteria only- DNA isolation)**

Prepare a 45 mg/ml solution of Lysozyme (approximately  $2.115 \times 10^6$  unit/ml) with the Gram-Positive Lysis Solution (GPLA) as the diluent, which is provided in the kit. Lysozyme solution should be freshly prepared prior to use.

Example: In order to make 1 ml of Lysozyme solution, dissolve 45 mg of lysozyme (provided) in 1 ml of Gram-Positive Lysis Solution (GPLA). Mix by pipetting or vortex to dissolve the lysozyme.

**NOTE:** Lysozyme dissolves readily by pipetting as opposed to vortexing. Excessive vortexing may cause foaming.

For each DNA preparation, 200  $\mu$ l of lysozyme solution is required. Make extra solution to account for pipetting error. The lysozyme solution should be preferably used on the day of preparation. If some Lysozyme stock solution is left, it can be stored at  $-20^{\circ}\text{C}$ .

10. **Prepare Lysozyme Solution (For Gram-negative and Gram-positive bacteria only- RNA isolation)**

Prepare a **20 mg/ml** solution of Lysozyme (approximately  $2.115 \times 10^6$  unit/ml) in TE Buffer (10mM Tris Cl, 1mM EDTA pH 8.0) (**not provided**). Lysozyme solution should be freshly prepared prior to use.

Example: In order to make 1ml of Lysozyme solution, dissolve 20mg of lysozyme (provided) in 1ml of TE Buffer (10mM Tris Cl, 1mM EDTA pH 8.0) (not provided). Pipette the mixture up and down or vortex to dissolve the lysozyme.

**NOTE:** Lysozyme dissolves readily by pipetting up and down as opposed to vortexing. Excessive vortexing may cause foaming.

For each DNA preparation, 100  $\mu$ l of lysozyme solution is required. Make extra solution to account for pipetting error. The lysozyme solution should be preferably used on the day of preparation. If some Lysozyme stock solution is left, it can be stored at  $-20^{\circ}\text{C}$ .

11.  **$\beta$ -mercaptoethanol ( $\beta$ -ME) must be added to RNA Lysis Solution (HRL) (DS0037) before use.**

$\beta$ -ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 $\mu$ l  $\beta$ -ME per 1ml Lysis Solution. Lysis Solution containing  $\beta$ -ME can be stored at room temperature (15-25 $^{\circ}\text{C}$ ) for up to 1 month.

12. **Bacterial cells should be harvested in log-phase of growth.**

(Inoculate a single colony in 30-50ml broth and incubate for 3 hours at desired temperature)

**RNase A enzyme treatment**

RNase A is a type of RNase that is commonly used in research. RNase A (e.g., bovine pancreatic ribonuclease A) is one of the sturdiest enzymes in common laboratory usage. It cleaves 3' end of unpaired C and U residues.

**Unit Definition for RNase A**

One unit of the enzyme causes an increase in absorbance of 1.0 at 260 nm when yeast RNA is hydrolyzed at 37 $^{\circ}\text{C}$  and pH 5.0. Fifty units are approximately equivalent to 1 Kunitz unit. It is completely free of DNases and proteases. The specific activity is 90 U/mg.

The product as supplied is stable at room temperature (15-25 $^{\circ}\text{C}$ ).

## Centrifugation

All centrifugation steps are carried out in conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with fixed angle rotor. The tubes provided with the kit are compatible with almost all laboratory centrifuges and rotors. All centrifugation steps are performed at room temperature and are given in g, the correct rpm can be calculated using the formula:

$$RPM = \sqrt{RCF/1.118} \times 10^5 r$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

## Specimen Handling and Collection

Collect human/animal cells, tissues, blood sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage. Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the tissue is at room temperature before beginning the protocol. After use, contaminated material must be sterilized by autoclaving before discarding.

## Types of Specimen

Clinical samples: human/ animal tissue, blood, cells

Animal samples: blood, cells

Bacterial cells (gram positive and gram negative)

## DNA ISOLATION FROM BLOOD

### Procedure

#### 1. Lysis

**NOTE: For Frozen blood:** To 200 µl of frozen blood pellet (kept on ice), add 200 µl of Lysis Solution (C1) (DS0010) and thaw the pellet with continuous pipetting. Proceed with step 2 for Proteinase K and RNase A treatment (optional). Incubate for 55°C for 10 minutes and then proceed to step 4 (Binding) of the protocol.

**NOTE:** We recommend freezing blood in 200 µl aliquots. When extracting DNA from frozen blood, it is very important that the blood should be kept on ice and directly mixed with Lysis Solution (C1). Do not allow the frozen blood pellet to thaw except when it is directly mixed with Lysis Solution (C1). This prevents release of apoptotic enzymes that can decrease the DNA yield drastically.

**NOTE:** If the sample is less than 200 µl, add the Resuspension Buffer (MS) to bring the volume upto 200 µl.

**NOTE: For blood sample stored at 2-8° or Room temperature (15-25°C) :**

2. Add 20 µl of the reconstituted Proteinase K solution (20 mg/ml) (**Refer to General Instructions Preparation**) into 2.0 ml capped collection tube containing 200 µl of the whole blood. Vortex for 10-15 seconds to ensure thorough mixing.

#### Optional RNase A treatment

If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (20 mg/ml) (DS0003). Vortex for 10-15 seconds and incubate for 2 minutes at room temperature (15-25°C).

3. **Lysis reaction**

Add 200 µl of the Lysis Solution (C1) (DS0010) to the sample, vortex thoroughly for a few seconds to obtain a homogenous mixture. Incubate at 55°C for 10 minutes and continue with step 4 (Binding).

**NOTE:** If cell clumps are visible, the sample can be mixed gently by pipetting to obtain a homogenous mixture.

4. **Prepare for Binding**

Add 200 µl of ethanol (96-100%) to the lysate obtained from the above steps for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting.

**NOTE:** A homogenous solution is essential.

5. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**

Transfer the lysate obtained from step 4 onto the HiElute Miniprep Spin Column (Capped) (in DBCA016 Collection Tube) provided. Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.

**NOTE:** Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.

6. **Prewash**

**(Prepare Prewash Solution Concentrate (PW) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Prewash Solution (PW) (DS0011) to the column and centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

7. **Wash**

**(Prepare Wash Solution Concentrate (WS) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge at  $12,000 - 16,000 \times g$  ( $\approx 13,000-16,000$  rpm) for 3 minutes to dry the column. Discard the flow-through liquid and spin the empty column for another minute at the same speed. Discard the collection tube containing the flow-through liquid and place the column in a new 2.0 ml uncapped collection tube.

**NOTE:** The column must be free of ethanol before eluting the DNA. The tube can be emptied and re-used for this additional centrifugation step.

8. **DNA Elution**

Pipette 100 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute to elute the DNA. Repeat the step again with another 100 µl of Elution Buffer (ET) for high yield of DNA.

**NOTE:** DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time; however, the overall DNA yield would be low. Storing DNA in water may cause acid hydrolysis. To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. Elution with volume less than 200µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

9. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

**Storage of the eluate with purified DNA:** The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

## **DNA ISOLATION FROM CULTURED CELLS**

### **Procedure**

#### **1. Harvest cells**

- **Attached cell cultures:** The cells can be detached using trypsin. Centrifuge upto  $5 \times 10^6$  cells for 5 minutes at  $300 \times g$  ( $\approx 1500$  rpm). Discard the culture medium and continue with step 2 of cultured cell.
- **Suspension cell cultures:** Centrifuge upto  $5 \times 10^6$  cells for 5 minutes at  $300 \times g$  [ $\approx 1500$  rpm]. Discard the culture medium completely and continue with step 2 of cultured cell.

**NOTE:** Cells can be harvested, by aliquoting in 2.0 ml microcentrifuge tubes and flash-freezing in liquid nitrogen, these can be stored at -70°C for several months before preparing DNA.

#### **2. Resuspend cells**

Resuspend the pellet obtained from step 1 of cultured cell, in 2ml capped collection tube 200  $\mu$ l of Resuspension Buffer (MS) (DS0090) and mix thoroughly. If previously frozen, allow the cell pellet to thaw slightly before resuspending. If residual RNA is not a concern, continue with step 3 of cultured cell.

#### **Optional RNase A treatment**

If RNA-free genomic DNA is required, add 20  $\mu$ l of RNase A Solution (DS0003), mix and incubate for 2 minutes at room temperature (15-25°C), then continue with step 3 of cultured cell.

#### **3. Lyse cells**

Add 20  $\mu$ l of the Proteinase K solution (20 mg/ml) (**Refer to General Instruction Preparation**) to the sample from step 1 of cultured cell, followed by 200  $\mu$ l of Lysis Solution (C1) (DS0010). Mix thoroughly by vortexing for about 15 seconds and incubate at 70°C for 10 minutes.

**NOTE:** A homogeneous mixture is essential for efficient lysis. Continue with step 4 (Binding).

#### **4. Prepare for Binding**

Add 200  $\mu$ l of ethanol (96-100%) to the lysate obtained from the above steps for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting.

**NOTE:** A homogenous solution is essential.

#### **5. Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**

Transfer the lysate obtained from step 4 onto the HiElute Miniprep Spin Column (Capped) (in DBCA016 Collection Tube) provided. Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.

**NOTE:** Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents onto the column.

6. **Prewash**

**(Prepare Prewash Solution Concentrate (PW) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Prewash Solution (PW) (DS0011) to the column and centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

7. **Wash**

**(Prepare Wash Solution Concentrate (WS) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge at  $12,000 - 16,000 \times g$  ( $\approx 13,000-16,000$  rpm) for 3 minutes to dry the column. Discard the flow-through liquid and spin the empty column for another minute at the same speed. Discard the collection tube containing the flow-through liquid and place the column in a new 2.0 ml uncapped collection tube.

**NOTE:** The column must be free of ethanol before eluting the DNA. The tube can be emptied and re-used for this additional centrifugation step.

8. **DNA Elution**

Pipette 100 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute to elute the DNA. Repeat the step again with another 100 µl of Elution Buffer (ET) for high yield of DNA.

**NOTE:** DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time; however, the overall DNA yield would be low. Storing DNA in water may cause acid hydrolysis. To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. Elution with volume less than 200µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

9. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

**Storage of the eluate with purified DNA:** The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

## **DNA ISOLATION FROM MAMMALIAN TISSUE**

### **Procedure**

1. **Prepare tissue**

Weigh up to 25 mg of tissue (or 10 mg of spleen, due to the high number of cells per given mass). To the weighed tissue, add approximately 5 ml of 0.8% saline. Homogenize the same with tissue lyser or homogenizer. Pellet down and discard the supernatant. Use the pelleted tissue for further lysis.

**NOTE:** If frozen tissue is used, allow it to thaw slightly before slicing but keep on ice in order to protect degradation.

**NOTE:** Tissue can be harvested, by aliquoting in capped 2.0 ml collection tubes and flash freezing in liquid nitrogen; these can be stored at -70°C for several months before preparing DNA.

2. **Digest tissue**

Add 180 µl of Resuspension Buffer (MS) (DS0090) and 20 µl of the Proteinase K solution (20 mg/ml) (**Refer to General Instruction Preparation**) to the tissue. Mix by vortexing. Incubate the sample at 55°C until the tissue is completely digested with no particles remaining. Mix by vortexing occasionally or use a shaking water bath. Digestion is usually complete in 2 to 4 hours. Vortex briefly after digestion is completed.

**Optional RNase A treatment**

If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (DS0003), mix and incubate for 2 minutes at room temperature (15-25°C), then continue with step 3 of Mammalian tissue.

3. **Lyse cells**

Add 200µl of Lysis Solution (C1) (DS0010) to the sample. Mix by vortexing thoroughly for 15 seconds. A homogeneous mixture is essential for efficient cell lysis. Incubate at 70°C for 10 minutes. Continue with step 4 (Binding).

4. **Prepare for Binding**

Add 200 µl of ethanol (96-100%) to the lysate obtained from the above steps for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting.

**NOTE:** A homogenous solution is essential.

5. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**

Transfer the lysate obtained from step 4 onto the HiElute Miniprep Spin Column (Capped) (in DBCA016 Collection Tube) provided. Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.

**NOTE:** Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.

6. **Prewash**

**(Prepare Prewash Solution Concentrate (PW) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Prewash Solution (PW) (DS0011) to the column and centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

7. **Wash**

**(Prepare Wash Solution Concentrate (WS) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge at  $12,000 - 16,000 \times g$  ( $\approx 13,000-16,000$  rpm) for 3 minutes to dry the column. Discard the flow-through liquid and spin the empty column for another minute at the same speed. Discard the collection tube containing the flow-through liquid and place the column in a new 2.0 ml uncapped collection tube.

**NOTE:** The column must be free of ethanol before eluting the DNA. The tube can be emptied and re-used for this additional centrifugation step.

## 8. DNA Elution

Pipette 100 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute to elute the DNA. Repeat the step again with another 100 µl of Elution Buffer (ET) for high yield of DNA.

**NOTE:** DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time; however, the overall DNA yield would be low. Storing DNA in water may cause acid hydrolysis. To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. Elution with volume less than 200µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

9. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

**Storage of the eluate with purified DNA:** The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

## **DNA ISOLATION FROM RODENT TAIL**

### **Procedure**

#### 1. Prepare rodent tails

Measure and cut a piece of fresh or frozen rodent tail. Before cutting, allow the frozen rodent tail to thaw slightly keep on ice in order to protect degradation. Do not use more than 0.6 cm (rat) or 1.2 cm (mouse) tail per preparation. Cut two (mouse) or one (rat) 0.5-0.6 cm lengths of tail and place them in a capped 2.0 ml collection tube. Continue with step 2.

**NOTE:** Rodent tails can be stored at -20°C for several months before preparing DNA.

#### 2. Digest tissue

Add 180 µl of Resuspension Buffer (MS) (DS0090) and 20 µl of the Proteinase K solution (20 mg/ml) (**Refer to General Instruction Preparation**) to the rodent tail. Mix by vortexing. Ensure that the tail is fully submerged. Incubate the sample at 55°C until the tail tissue is completely digested. Some particles (bone and hair) may remain. Mix by vortexing occasionally or use a shaking water bath, during incubation for more rapid digestion. Digestion is usually complete in 3 to 6 hours. Vortex briefly after digestion is complete. If residual RNA is not a concern, continue with step 3 Rodent tail.

#### **Optional RNase A treatment**

If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (DS0003), mix and incubate for 2 minutes at room temperature (15-25°C), then continue with step 3 of Rodent tail.

#### 3. Lysis reaction

Add 200 µl of Lysis Solution (C1) (DS0010) to the sample. Vortex thoroughly for 15 seconds. A homogeneous mixture is essential for efficient cell lysis. Incubate at 70°C for 10 minutes. Continue with step 4 (Binding).

#### 4. Prepare for Binding

Add 200 µl of ethanol (96-100%) to the lysate obtained from the above steps for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting.

**NOTE:** A homogenous solution is essential.

5. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**

Transfer the lysate obtained from step 4 onto the HiElute Miniprep Spin Column (Capped) (in DBCA016 Collection Tube) provided. Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.

**NOTE:** Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.

6. **Prewash**

**(Prepare Prewash Solution Concentrate (PW) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Prewash Solution (PW) (DS0011) to the column and centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

7. **Wash**

**(Prepare Wash Solution Concentrate (WS) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge at  $12,000 - 16,000 \times g$  ( $\approx 13,000-16,000$  rpm) for 3 minutes to dry the column. Discard the flow-through liquid and spin the empty column for another minute at the same speed. Discard the collection tube containing the flow-through liquid and place the column in a new 2.0 ml uncapped collection tube.

**NOTE:** The column must be free of ethanol before eluting the DNA. The tube can be emptied and re-used for this additional centrifugation step.

8. **DNA Elution**

Pipette 100 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute to elute the DNA. Repeat the step again with another 100 µl of Elution Buffer (ET) for high yield of DNA.

**NOTE:** DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time; however, the overall DNA yield would be low. Storing DNA in water may cause acid hydrolysis. To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. Elution with volume less than 200µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

9. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

**Storage of the eluate with purified DNA:** The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

## **DNA ISOLATION FROM GRAM- NEGATIVE BACTERIA**

### **Procedure**

#### **1. Harvesting of cells**

Pellet 1.5 ml of bacterial broth culture in 2.0ml capped collection tube by centrifuging for 2 minutes at 12,000-16,000 x g ( $\approx$ 13,000-16,000 rpm). Remove the culture medium completely and discard.

**NOTE:** If bacteria are grown in rich media such as Terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml in order to avoid overloading of the HiElute Miniprep Spin Column (Capped).

#### **2. Resuspend cells**

Resuspend the pellet thoroughly in 180 $\mu$ l Resuspension Buffer (MS) (DS0090).

#### **3. Prepare for cell lysis**

Add 20  $\mu$ l of the Proteinase K solution (20mg/ml) (**Refer to General Instruction Preparation**) to the sample. Mix and incubate for 30 minutes at 55°C. If residual RNA is not a concern, continue with step 4 of gram negative bacteria.

#### **Optional RNase A treatment**

If RNA-free genomic DNA is required, add 20  $\mu$ l of RNase A Solution (DS0003), mix and incubate for 5 minutes at room temperature (15-25°C), then continue with step 4 of gram negative bacteria.

#### **4. Lyse cells**

Add 200  $\mu$ l of Lysis Solution (C1) (DS0010), vortex thoroughly (about 15 seconds) and incubate at 55°C for 10 minutes. Continue with step 5 (Binding).

**NOTE:** A homogeneous mixture is essential for efficient lysis.

#### **5. Prepare for Binding**

Add 200  $\mu$ l of ethanol (96-100%) to the lysate obtained from the above steps for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting.

**NOTE:** A homogenous solution is essential.

#### **6. Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**

Transfer the lysate obtained from step 5 onto the HiElute Miniprep Spin Column (Capped) (in DBCA016 Collection Tube) provided. Centrifuge at  $\geq$ 6,500 x g ( $\approx$ 10,000 rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.

**NOTE:** Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.

#### **7. Prewash**

**(Prepare Prewash Solution Concentrate (PW) as indicated in General Preparation Instructions)**

Add 500  $\mu$ l of diluted Prewash Solution (PW) (DS0011) to the column and centrifuge at  $\geq$ 6,500 x g ( $\approx$ 10,000 rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

#### **8. Wash**

**(Prepare Wash Solution Concentrate (WS) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge at 12,000 – 16,000 x g (≈13,000-16,000 rpm) for 3 minutes to dry the column. Discard the flow-through liquid and spin the empty column for another minute at the same speed. Discard the collection tube containing the flow-through liquid and place the column in a new 2.0 ml uncapped collection tube.

**NOTE:** The column must be free of ethanol before eluting the DNA. The tube can be emptied and re-used for this additional centrifugation step.

#### 9. DNA Elution

Pipette 100 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to elute the DNA. Repeat the step again with another 100 µl of Elution Buffer (ET) for high yield of DNA.

**NOTE:** DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time; however, the overall DNA yield would be low. Storing DNA in water may cause acid hydrolysis. To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. Elution with volume less than 200µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

10. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

**Storage of the eluate with purified DNA:** The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

## **DNA ISOLATION FROM GRAM- POSITIVE BACTERIA**

### **Procedure**

1. Prepare Lysozyme Solution using Lysozyme from chicken egg white, which is provided in the kit.

Prepare a 45 mg/ml stock solution of lysozyme as described under General Preparation Instructions. 200 µl of Lysozyme Solution is required per isolation procedure. Prepare extra solution to account for pipetting error.

**NOTE:** (Optional) For higher yields - If working with *Staphylococcus* species, supplement the Lysozyme Solution with 200 units/ml of lysostaphin (not provided). For *Streptococcus* species, supplement the Lysozyme Solution with 250 units/ml of mutanolysin (not provided).

2. **Harvest Cells**

Pellet 1.5 ml of bacterial broth culture in 2.0ml capped collection tube by centrifuging for 2 minutes at 12,000-16,000 x g (≈13,000-16,000rpm). Remove the culture medium completely and discard.

**NOTE:** If bacteria are grown in rich media such as Terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml in order to avoid overloading of the HiElute Miniprep Spin Column (Capped).

3. **Resuspend cells**  
Resuspend the pellet thoroughly in 200 µl of lysozyme solution (prepared in step 1 of gram positive bacteria) and incubate for 30 minutes at 37°C.
4. **Lyse cells**  
Add 20µl of the Proteinase K solution (20 mg/ml) (**Refer to General Instruction Preparation**) to the sample. If residual RNA is not a concern continue with step 5 of gram positive bacteria.  
  
**Optional RNase A treatment**  
If RNA- free genomic DNA is required, add 20 µl of RNase A Solution (DS0003), mix and incubate for 5 minutes at room temperature (15-25°C), then continue with step 5 of gram positive bacteria.
5. Add 200 µl of Lysis Solution (C1) (DS0010). Vortex thoroughly for few seconds and incubate at 55°C for 10 minutes, then continue with step 6 (Prepare for binding).  
  
**NOTE:** A homogeneous mixture is essential for efficient lysis.
6. **Prepare for Binding**  
Add 200 µl of ethanol (96-100%) to the lysate obtained from the above steps for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting.  
  
**NOTE:** A homogenous solution is essential.
7. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**  
Transfer the lysate obtained from step 6 onto the HiElute Miniprep Spin Column (Capped) (in DBCA016 Collection Tube) provided. Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.  
  
**NOTE:** Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.
8. **Prewash**  
**(Prepare Prewash Solution Concentrate (PW) as indicated in General Preparation Instructions)**  
Add 500 µl of diluted Prewash Solution (PW) (DS0011) to the column and centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.
9. **Wash**  
**(Prepare Wash Solution Concentrate (WS) as indicated in General Preparation Instructions)**  
Add 500 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge at  $12,000 - 16,000 \times g$  ( $\approx 13,000-16,000$  rpm) for 3 minutes to dry the column. Discard the flow-through liquid and spin the empty column for another minute at the same speed. Discard the collection tube containing the flow-through liquid and place the column in a new 2.0 ml uncapped collection tube.  
  
**NOTE:** The column must be free of ethanol before eluting the DNA. The tube can be emptied and re-used for this additional centrifugation step.
10. **DNA Elution**  
Pipette 100 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at  $\geq 6,500 \times g$

g ( $\approx 10,000$  rpm) for 1 minute to elute the DNA. Repeat the step again with another 100  $\mu$ l of Elution Buffer (ET) for high yield of DNA.

**NOTE:** DNA elution can also be performed in single step by the addition of 200  $\mu$ l of Elution Buffer (ET) at a time; however, the overall DNA yield would be low. Storing DNA in water may cause acid hydrolysis. To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. Elution with volume less than 200  $\mu$ l increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

11. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

**Storage of the eluate with purified DNA:** The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

### **Precautions to be taken while handling RNA**

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.
2. Use sterile, disposable plasticware and autoclavable pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipments.
3. Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH, 1mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.
4. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for four or more hours before use. Alternatively glassware can be treated with DEPC (Diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 min to eliminate residual DEPC.
5. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
6. Solutions (water and other solutions) should be treated with 0.1% DEPC.

## RNA ISOLATION FROM ANIMAL CELLS

### Procedure

#### 1. Harvest the cells according to step a, b, or c

##### a. Cells grown in suspension (do not use more than $1 \times 10^7$ cells):

Pellet up to  $1 \times 10^7$  cells by centrifuging for 5 minutes at  $300 \times g$  ( $\approx 1500$  rpm) in a Collection tube (not supplied). Discard the culture medium completely and continue with step 2 of Lysis reaction.

**NOTE:** Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane resulting in lower RNA yield.

##### b. Cells grown in a monolayer (do not use more than $1 \times 10^7$ cells):

Direct lysis of the cells can be done in the cell-culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell-culture flasks should always be trypsinized.

##### To trypsinize and collect cells:

Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.1–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at  $300 \times g$  for 5 minutes. Completely aspirate the supernatant, and proceed to step 2 of Lysis reaction.

**NOTE:** Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane resulting in lower RNA yield.

##### c. To lyse cells directly in culture dish:

Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2 of Lysis reaction.

**NOTE:** Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane resulting in lower RNA yield.

#### 2. Lysis reaction

##### a. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of RNA Lysis Solution (HRL) (DS0037) (see the table below). Vortex or pipet to mix, and continue with step 3.

**NOTE:** Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields. Ensure that  $\beta$ -ME is added to RNA Lysis Solution (HRL) before use.

Number of pelleted cells	RNA Lysis Solution (HRL)
$<5 \times 10^6$	350 $\mu$ l
$5 \times 10^6 - 1 \times 10^7$	600 $\mu$ l

##### b. For Cells grown in a monolayer, add appropriate volume of RNA Lysis Solution (HRL) (see the table below) to the cell culture dish. Collect cell lysate into a collection tube (not supplied). Vortex or pipet to mix and ensure that no cell clumps are visible before proceeding to step 3.

**NOTE:** Regardless of the cell number, use the Lysis Solution volumes indicated to completely cover the surface of the dish. Ensure that  $\beta$ -ME is added to RNA Lysis Solution (HRL) before use.

Dish diameter	RNA Lysis Solution (HRL)
< 6 cm	350 $\mu$ l
6-10 cm	600 $\mu$ l

3. **Homogenize the lysate**

If processing  $\leq 1 \times 10^5$  cells, homogenize by vortexing for 1 minute. After homogenization, proceed to step 4.

**NOTE:** Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the HiElute Miniprep Spin Column (Capped). Homogenization with a HiShredder generally results in higher RNA yields.

4. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2 ml uncapped collection tube, and centrifuge for 2 minutes at 13,000 X g (14,000 rpm). Prepare for binding step.

5. **Prepare for binding:**

Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.

**NOTE:** A precipitate may be visible after addition of ethanol. This does not affect the procedure.

6. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**

Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow-through.

**NOTE:** If the volume exceeds 700  $\mu$ l, load aliquots successively onto the HiElute Miniprep Spin column and centrifuge as above. Discard the flow-through after each centrifugation step.

**Optional: On Column DNase digestion**

**Generally, DNase I digestion is not required since the solutions of this kit efficiently removes almost all the DNA without DNase treatment.** However, further DNase treatment may be necessary for certain RNA applications that are sensitive to small amounts of DNA (e.g. TaqMan RT-PCR analysis with a low abundant target). DNA can also be removed by DNase digestion.

Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column as indicated above. Instead of continuing with the Prewash Solution (RW1) in step 7, follow steps a–d below.

- a. Pipet 350  $\mu$ l of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow through and reuse the collection tube in step 6c.
- b. Add 10  $\mu$ l of DNase I Solution to 70  $\mu$ l of DNase Digest Buffer. Mix by inversion. Do not vortex.
- c. Add 80  $\mu$ l of DNase I/Digest Buffer mixture directly onto the HiElute Miniprep Spin Column (Capped). Incubate at room temperature for 15 minutes.

- d. Pipet 350  $\mu$ l of Prewash Solution (RW1) into the HiElute Miniprep Spin column , and centrifuge for 15 seconds at centrifuge at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow-through and continue with the step 8.

**Or**

**Alternatively, proceed with RNA isolation protocol without DNase I treatment (continue with step 7).**

**7. Prewash Solution (RW1)**

Add 700  $\mu$ l of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

8. Pipet 500  $\mu$ l of Wash Solution (WS) (DS0012). Close the tube gently, and centrifuge for 1 minute at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to wash the column. Discard the flow-through.

**NOTE:** Wash Solution (WS) is supplied as a concentrate. Ensure that ethanol is added to Wash Solution Concentrate (WS).

9. Add another 500  $\mu$ l of Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

**Optional:** Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

**10. RNA Elution**

Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30-50  $\mu$ l Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to elute.

- If the expected RNA yield is  $> 30 \mu\text{g}$ , repeat the elution step as described with a second volume of RNase-Free Water. Elute into the same collection tube.
11. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA storage.

**NOTE:** To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate. The yield will be 15-30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

**Storage of the eluate with purified RNA:** The eluate contains pure RNA, recommended to be stored at lower temperature ( $-80^{\circ}\text{C}$ ). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

## **RNA ISOLATION FROM ANIMAL TISSUES**

### **Procedure**

1. **Fresh or frozen (up to 30 mg) tissue.**

Immediately place the weighed (fresh or frozen) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Allow the liquid nitrogen to evaporate. Do not allow the samples to thaw. Proceed with the lysis.

2. **Lysis reaction**

Add appropriate volume of RNA Lysis Solution (HRL) (DS0037) (see the table below). Mix properly and transfer it to an RNase free 2.0 ml capped collection tube. Vortex or pipet to mix.

<b>Amount of starting material</b>	<b>RNA Lysis Solution (HRL)</b>
<20 mg	350 $\mu$ l
20 mg to 30 mg	600 $\mu$ l

**NOTE:** Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields. Ensure that  $\beta$ -ME is added to RNA Lysis Solution (HRL) before use.

3. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2 ml uncapped collection tube, and centrifuge for 2 min at full speed. Continue with binding.

4. **Prepare for binding:**

Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.

**NOTE:** A precipitate may be visible after addition of ethanol. This does not affect the procedure.

5. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**

Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow-through.

**NOTE:** If the volume exceeds 700  $\mu$ l, load aliquots successively onto the HiElute Miniprep Spin column and centrifuge as above. Discard the flow-through after each centrifugation step.

**Optional: On Column DNase digestion**

**Generally, DNase I digestion is not required since the solutions of this kit efficiently removes almost all the DNA without DNase treatment.** However, further DNase treatment may be necessary for certain RNA applications that are sensitive to small amounts of DNA (e.g. TaqMan RT-PCR analysis with a low abundant target). DNA can also be removed by DNase digestion.

Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column as indicated above. Instead of continuing with the Prewash Solution (RW1) in step 6, follow steps a–d below.

a. Pipet 350  $\mu$ l of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow through and reuse the collection tube in step 6c.

- b. Add 10  $\mu$ l of DNase I Solution to 70  $\mu$ l of DNase Digest Buffer. Mix by inversion. Do not vortex.
- c. Add 80  $\mu$ l of DNase I/Digest Buffer mixture directly onto the HiElute Miniprep Spin Column (Capped). Incubate at room temperature for 15 minutes.
- d. Pipet 350  $\mu$ l of Prewash Solution (RW1) into the HiElute Miniprep Spin column , and centrifuge for 15 seconds at centrifuge at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the flow-through and continue with the step 7.

Or

**Alternatively, proceed with RNA isolation protocol without DNase I treatment (continue with step 6).**

**6. Prewash Solution (RW1)**

Add 700  $\mu$ l of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

7. Pipet 500  $\mu$ l of Wash Solution (WS) (DS0012). Close the tube gently, and centrifuge for 1 minute at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to wash the column. Discard the flow-through.

**NOTE:** Wash Solution (WS) is supplied as a concentrate. Ensure that ethanol is added to Wash Solution Concentrate (WS).

8. Add another 500  $\mu$ l of Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

**Optional:** Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at  $13,000 \times g$  (14,000 rpm) for 1 minute.

**9. RNA Elution**

Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30-50  $\mu$ l Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) to elute.

- If the expected RNA yield is  $> 30 \mu\text{g}$ , repeat the elution step as described with a second volume of RNase-Free Water. Elute into the same collection tube.

10. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA storage.

**NOTE:** To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate. The yield will be 15-30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

**Storage of the eluate with purified RNA:** The eluate contains pure RNA, recommended to be stored at lower temperature ( $-80^{\circ}\text{C}$ ). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

## RNA ISOLATION FROM BACTERIA (GRAM- NEGATIVE AND GRAM- POSITIVE)

### Procedure

#### 1. Harvest and Resuspend the cells

Harvest bacteria in capped 2.0ml collection tube by centrifugation at 10,000 X g ( $\geq 13,000$  rpm) for 5 min at 4°C. (Do not use more than  $1 \times 10^7$  bacteria). Remove the culture medium and discard.

Resuspend the bacterial pellet in 100 $\mu$ l of lysozyme containing TE Buffer and mix well by gentle vortexing till no cell clumps are visible.

(Prepare lysozyme containing TE Buffer as indicated in General Preparation Instructions)

Incubate the resuspended cells at 37°C for 30 minutes. Incubate on shaker or vortex 20 seconds for every 2 minutes during incubation

#### 2. Lysis

Add 350 $\mu$ l or 600 $\mu$ l of RNA Lysis Solution (HRL) (DS0037) to the sample. Vortex or pipet to mix.

**NOTE:** If insoluble material is visible, centrifuge for 2 minutes at 13,000rpm at room temperature (15-25°C), and use only the supernatant in the subsequent steps.

**NOTE:** Ensure that  $\beta$ -ME is added to RNA Lysis Solution (HRL) before use.

Number of pelleted cells	RNA Lysis Solution (HRL)
$< 5 \times 10^6$	350 $\mu$ l
$5 \times 10^6 - 1 \times 10^7$	600 $\mu$ l

3. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2ml collection tube, and centrifuge at ( $\geq 13,000$  rpm) for 2 minutes at room temperature (15-25°C). Collect the flow-through in the fresh collection tube (not provided) and proceed for binding step.

#### 4. Prepare for binding:

Add 280 $\mu$ l of ethanol (96-100%) to the cleared lysate, and mix immediately by pipetting. Do not centrifuge.

**NOTE:** A precipitate may form after the addition of ethanol, but this will not affect the procedure.

#### 5. Load Lysate in HiElute Miniprep Spin Column (DBCA02)

Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 1 minute at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) at room temperature (15-25°C). Discard the flow-through.

**NOTE:** If the volume exceeds 700 $\mu$ l, load aliquots successively onto the HiElute Miniprep Spin column and centrifuge as above. Discard the flow-through after each centrifugation step.

#### 6. Optional: On Column DNase digestion

**Generally, DNase I digestion is not required since the solutions of this kit efficiently removes almost all the DNA without DNase treatment.** However, further DNase treatment may be necessary for certain RNA applications that are sensitive to small amounts of DNA (e.g. TaqMan RT-PCR analysis with a low abundant target). DNA can also be removed by DNase digestion.

Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column as indicated above. Instead of continuing with the Prewash Solution (RW1) in step 7, follow steps a–d below.

- a. Pipet 350µl of Pre Wash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column, and centrifuge for 1 minute at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) at room temperature (15-25°C). Discard the flow through and reuse the collection tube in step 6c.
- b. Add 10µl of DNase I Solution to 70µl of DNase Digest Buffer. Mix by inversion. Do not vortex.
- c. Add 80µl of DNase I/ Digest Buffer mixture directly onto the HiElute Miniprep Spin Column. Incubate at room temperature (15-25°C) for 15 minutes.
- d. Pipet 350µl of Pre Wash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 1 minute at centrifuge at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) at room temperature (15-25°C). Discard the flow through and continue with the step 8.

**Or**

**Alternatively, proceed with RNA isolation protocol without DNase I treatment (continue with step 7).**

**7. Pre Wash**

Add 700µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column centrifuge at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) for 1 minute at room temperature (15-25°C). Discard the flow-through. Reuse the collection tube in step 8.

8. Transfer the HiElute Miniprep Spin column into a 2ml collection tube. Pipet 500µl of diluted Wash Solution (WS) (DS0012) (**Refer General Preparation Instructions**). Close the tube gently, and centrifuge for 1 minute at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) at room temperature (15-25°C) to wash the column. Discard the flow-through.
9. Add another 500µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column. Close the tube gently, and centrifuge for 2 minutes at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) at room temperature (15-25°C) to dry the membrane.
10. Place the column in a new 2ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at  $\geq 10000 \times g$  ( $\geq 13,000$  rpm) at room temperature (15-25°C) for 1 minute.

**11. RNA Elution**

Transfer the HiElute Miniprep Spin column (capped) to a new uncapped 2ml collection tube. Pipet 30-50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column. Close the tube gently, and centrifuge for 1 minute at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm) at room temperature (15-25°C) to elute.

If the expected RNA yield is  $> 30 \mu\text{g}$ , repeat the elution step (step 10) as described with a second volume of RNase-Free Water. Elute into the same collection tube.

12. Transfer the eluate in to new capped 2.0ml collection tube for longer storage.

**NOTE:** To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate. The yield will be 15-30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

**Storage of the eluate with purified RNA:** The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

### Warning and Precautions

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

### Limitations

The yield of DNA and RNA depends upon the type and the volume of starting material used.

### Performance and Evaluation

Performance of the kit is expected when the kit is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.

### Quality Control

Type of Sample	DNA Yield	DNA Purity	RNA Yield	RNA Purity
Human blood	4-12 µg	1.6-1.9	-	-
Animal tissue	-	-	upto 100 µg	1.8-2.1

### Trouble shooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1.	Presence of cell clumps / colored residue on the spin column after washing	Inefficient cell lysis due to improper mixing of the lysis buffer with the sample.	The sample and the Lysis Solution (C1) should be mixed thoroughly by pulse-vortexing.
		Due to decreased Proteinase K activity	Do not add Proteinase K directly to the Lysis Solution (C1). Ensure that the stock solution is stored as indicated.
2.	Poor or low genomic DNA recovery	Lysate/ethanol mixture is not homogenous	In order to obtain a homogenous solution, mix thoroughly by gentle pipetting before adding to the HiElute Miniprep Spin Column (Capped).
		DNA elution is improper	Ensure that the DNA elution is in 200 µl of Elution Buffer (ET). To improve the DNA yield, incubate for 5 minutes at room temperature (15-25°C) after it is added to the column.
		Eluate contains residual ethanol from wash	Remove ethanol from the second wash completely before eluting the DNA. Spin for an additional minute to dry the membrane completely. In order to avoid the interference of ethanol, fresh tube can be used for elution.

		Use of water instead of Elution Buffer for elution of DNA	Elution Buffer (ET) is recommended for optimal yield and storage of the genomic DNA. If water is used instead of the elution buffer the pH should be at least 7.0, to avoid acidic conditions, which may cause acid hydrolysis of DNA when stored for long periods of time.
			<b>NOTE:</b> Only DNase/RNase and Protease free water should be used for eluting DNA.
		Wash Solution Concentrate was not diluted before use	Ensure that the Wash Solution concentrate is properly diluted with ethanol as per general preparation instructions.
3.	Purity of the DNA is lower than expected ( $A_{260}/A_{280}$ ratio is less)	Background reading is high due to silica fines	Spin the DNA sample at maximum speed for 1 minute and use the supernatant to repeat the absorbance readings.
		Eluate was diluted in water for absorbance measurement	Use the Elution Buffer (ET) provided with the kit.
		Purification is incomplete due to column overloading or inadequate lysis	Reduce the initial volume of the sample or increase the lysis time while monitoring the lysis visually.
4.	$A_{260}/A_{280}$ ratio is too high	RNA contamination	RNase A treatment should be included in future isolations or the final product can be treated with RNase A and re-purified.
5.	Shearing of genomic DNA	Improper handling of genomic DNA	All pipetting steps should be executed as gently as possible. Wide orifice pipette tips are recommended to eliminate shearing of the DNA to a large extent. If the isolated DNA is to be used for PCR, instead of vortexing, mix with gentle pipetting or invert until homogenous. This reduces shearing of DNA considerably.
		Sample is old, degraded, or has undergone repeated freeze/ thaw cycles	Fresh cells, tissues, rodent tail, whole blood should be used immediately. Old material may yield degraded DNA in the eluate. Cells and tissues can be frozen in liquid nitrogen and stored at $-70^{\circ}\text{C}$ until needed. Rodent tails can be stored at $-20^{\circ}\text{C}$ for several weeks or $-70^{\circ}\text{C}$ for several months. Whole blood can be stored at $4^{\circ}\text{C}$ for up to 12 hours.
		Cells are old	Cells grown for a longer time period

			may lyse prematurely when subjected to cell wall lysing enzymes, which may result in the release of endogenous nucleases and subsequent DNA degradation.
6.	Downstream applications are inhibited	Traces of ethanol present in the final genomic DNA preparation	After the washing steps, the eluate should not come in contact with the column. Spin the column for 1 minute at maximum speed (12,000-16,000 x g) if necessary, after emptying the collection tube.
		Salt is carried over in the final genomic DNA preparation	The column should be transferred to a new 2.0 ml collection tube before adding the wash solution.
7.	Clogged HiElute Miniprep Spin Column (Capped)	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		Tissues: Centrifugation before adding ethanol not performed	Centrifuge the lysate before adding ethanol and use only this supernatant in subsequent steps. Pellets contain cell debris can clog the HiElute Miniprep Spin Column (Capped).
		Centrifugation temperature is too low	The centrifugation temperature should be 20 – 25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol containing lysate to 37°C before transferring it to the column.
8.	Low RNA Yield	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material.
		RNA still bound to HiElute Miniprep Spin Column	Repeat RNA elution, but incubate the HiElute Miniprep Spin Column for 10 mins at room temperature (15-25°C) with Elution solution (RNase free water) before centrifuging.
		Ethanol carryover	During the second wash with Wash Solution (WS) be sure to centrifuge at 2 min at $\geq 8000 \times g$ ( $\geq 10,000$ rpm) to dry the column. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow through otherwise carryover of ethanol will occur. To eliminate any

			chance of possible ethanol, centrifuge the column for another minute at full speed.
		Incomplete removal of cell culture medium.	When processing cultured cells ensure complete removal of the cell culture medium after harvesting cells.

### Safety Information

The HiPurA® DNA/ RNA Purification Kit is for laboratory use only, not for drug, household or other uses. The Lysis Solution (C1) contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

### Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

### Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to [mb@himedialabs.com](mailto:mb@himedialabs.com).

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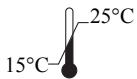
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