Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Pre-heat DEPC-water to 70° C prior to elution. • Incubate column for 10 min with water prior to centrifugation.
	Column is overloaded	• Reduce quantity of starting material.
Clogged column	Incomplete homogenization	• Completely homogenize sample. • Increase centrifugation time. • Reduce amount of starting material
Degraded RNA	Source	• Freeze starting material quickly in liquid nitrogen. • Do not store tissue culture cells prior to extraction unless they are lysed first. • Follow protocol closely, and work quickly.
	RNase contamination	• Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	• Ensure Wash Buffer II Concentrate has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • 1 X Wash Buffer II must be stored and used at room temperature. • Repeat wash with Wash Buffer II.
DNA contamination		• Digest with RNase-free DNase and inactivate at 75°C for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or water	• DEPC-treated water is acidic and can dramatically lower Abs260 values. Use TE buffer to dilute RNA prior to spectrophotometric analysis.

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Revised August 2006

Since HiBind® RNA resin and spin-column technology actually removes most of DNA without the DNase treatment, it is not necessary to do DNase digestion for most downstream applications. However, certain sensitive RNA applications might require further DNA removal. Following steps provide on-membrane DNase I digestion:(see DNase I cat.# E1091for detail information)

1. Follow the standard protocol until the samples completely pass through the HiBind RNA column. Prepare the following:

2. For each HiBind® RNA column, prepare the DNase I digestion reaction mix as follows:

OBI DNase I Digestion Buffer	73.5 µl
RNase-free DNase I (20 Kunitz unites/µl)	1.5 µl
Total volume	75 µl

Note:
a. DNase I is very sensitive for physical denaturaion, so do not vortex this DNase I mixture. Mix gently by inverting the tube. Prepare the fresh DNase I digestion mixture before RNA isolation.
b. OBI DNase I digestion buffer is supplied with OBI RNase-free Dnase set.
c. Standard Dnase buffers are not compatible with on-membrane Dnase digestion.

3. Pipet 75 µl of the DNase I digestion reaction mix directly onto the surface of HiBind® RNA resin in each column. Make sure to pipet the Dnase I digestion mixture directly onto the membrane. Dnase I digestion will not be complete if some of the mix stick to the wall or the O-ring of the HiBind® RNA column.

4. Incubate at room temperature(25-30°C) for 15 minutes

5. Place column into a 2ml centrifuge tube, and add 400µl RWC Wash Bufffer. Place the column at room temperature for 5 minutes. Centrifuge at 10,000 x g for 30 seconds and discard flow-through. Reuse the collection tube in next step.

6. Continue the wash and elution steps by following standard protocol.

DNA Contamination

Generally HiBind® RNA spin column technology will efficiently removes most of the

Kit Contents

Product Number	R6831-00	R6831-01	R6831-02
Purification times	5 preps	50 preps	200 preps
MicroElute ® RNA Columns	5	50	200
2 mL Collection Tubes	15	150	600
TRK Lysis Buffer	5 mL	25 mL	100 mL
RWC Wash Buffer	5 mL	50 mL	200 mL
RWB Wash Buffer	2 mL	12 mL	4 X 12 mL
DEPC-ddH_2O	1 mL	20 mL	60 mL
Carrier RNA	150 ug	1 mg	4X 1 mg
Linear Acrylamide	50 µl	250 µl	1ml
Instruction Manual	1	1	1

Before Starting

IMPORTANT	
	RWB Wash Buffer must be diluted with absolute ethanol before use and store at room temperature.
	R6831-00 Add 8 mL absolute ethanol (96-100%)
	R6831-01 Add 48 mL absolute ethanol (96-100%)
	R6831-02 Add 48 mL absolute ethanol to each bottle
	Carrier RNA must be dissolved with DEPC Water before use and **aliquot into adequate portions. Store at -20°C.**
	R6831-00 Add 150ul DEPC water
	R6831-01 Add 1 ml DEPC water
	R6831-02 Add 1 ml DEPC water to each bottle

- ♦ Under cool ambient conditions, crystals may form in TRK Lysis Buffer. This is normal and the bottle should be warmed to redissolve the salt.
- ♦ 2-mercaptoethanol (ß-mercaptoethanol) is the key in denaturing RNases and must be added to an aliquot of TRK Lysis Buffer before use. Add 20 µl of 2-mercaptoethanol per 1 mL of TRK Lysis Buffer. This mixture can be stored for 1 week at room temperature.
- ♦ All centrifugation steps must be carried out at 22°C-25°C.

add 0.5 volume of absolute ethanol (96-100%). Mix throughly by pipetting or vortexing.

8. **Place HiBind® MicroElute® RNA column into a 2 ml collection tube (supplied with kit) and apply the mixture from step 7 (including any precipitate) to the column.** Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through and collection tube.

9. **Place column in a clean 2 ml collection tube, and add 400 μl of RWC Wash Bufffer.** Centrifuge at 10, 000 x g for 30 seconds. Discard flow-through and re-use the collection tube.

10. ***DNase I Digestion (Optional): This is point to start On-membrane DNase I digestion. (See detail procedure on page 14)***.

11. **Place column in the same 2 ml collection tube and add 500 μl RWB Wash Buffer diluted with ethanol.** Centrifuge at 10, 000 x g for 30 seconds. Discard the flow-through and re-use the collection tube. Reuse the collection tube in step 12.

    Note: RWB Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for instructions

12. **Wash column with a second 500 μl of RWB Wash Buffer as in step 11.** Centrifuge for 30 seconds at 10,000 x g and discard flow-through. **Then with the collection tube empty, centrifuge the column for 2 min at full speed (≥13,000 x g) to completely dry the HiBind® matrix.**

13. **Elution of RNA. Transfer the column to a clean 1.5 mL microfuge tube (not supplied with kit) and elute the RNA with 15-20 μl of DEPC-treated water (supplied with kit).** Make sure to add water directly onto column matrix. Let it sit at room temperature for 2 minutes. Centrifuge 1 min at maximum speed.

    RNA may be eluted with a smaller (<15μl) volume of water to get higher RNA concentration. While reduced elution volume decrease total RNA yield. The total yield will be 20-30% less when the elution volume is less than 10μl.

E. RNA Purified from Enzymatic Reactions

MicroElute ® Total RNA Kit Protocols

A. Total RNA Isolation From Laser dissected samples

Materials supplied by user:

- 2-mercaptoethanol (14.3M)
- 70% ethanol in DEPC-treated sterile distilled water
- Sterile RNase-free pipette tips and microcentrifuge tubes
- Disposable latex gloves

Procedure:

1. **Collect the sample into a 1.5 centrifuge tube contains 65 μl or 300 μl TRK Lysis Buffer.** Remember to add 20 μl of 2-mercaptoethanol per 1 mL of TRK Lysis Buffer before use.

2. **Adjust the sample volume to 75 μl or 350 μl with TRK Lysis Buffer.** When process small amount of cells (≤5000 cells). Add 3 μl of linear acrylamide and 1 μl of Carrier RNA to the lysate before homogenization.

3. **Mix the sample throughly by vortexing for 30 seconds to homogenize the sample.**

4. **Add 1 volume (75 μl or 350 μl) of 70% ethanol to the homogenized lysate.** Mix well by pipetting or vortexing.

5. **Apply the mixture from step 4 onto MicroElute® RNA Column.** The maximum capacity of the spin cartridge is 750 μl. A precipitate may form on addition of ethanol. Vortex and add the entire mixture to the column. With the spin column inside a 2ml collecting tube (supplied with kit), centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow-through and proceed to step 6.

6. **Place column in a clean 2ml collection tube (supplied), and add 400 μl RWC Wash Buffer**. Centrifuge as above and discard flow-through. Reuse the collection tube for next step.
   Note: This the starting point if on-membrane Dnase I digestion (page 14) is desired.

7. **Place column in the same 2 ml collection tube, add 500 μl RWB Wash Buffer diluted with ethanol.** Centrifuge as above and discard the flow-through.

   Note: RWB Wash Buffer Concentrate must be diluted with absolute** ethanol **before use. Refer to label on bottle for instructions.

8. **Wash column with a second 500 μl of RWB Wash Buffer as in step 7.**

8. **DNase I Digestion (Optional):** this is point to start On-membrane DNase I digestion. (See detail procedure on page 14).

9. **Place column in the same 2ml collection tube and add 500 μl RWB Wash Buffer diluted with absolute ethanol.** Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through and re-use the collection tube.

   Note: RWB Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for instructions

10. **Wash column with a second 500 μl of RWB Wash Buffer as in step 9**. Centrifuge at 10,000 x g and discard flow-through. **Then with the collection tube empty, centrifuge the column for 2 min at full speed (≥ 13,000 x g) to completely dry the HiBind® matrix.**

11. **Elution of RNA. Transfer the column to a clean 1.5 mL microfuge tube (not supplied with kit) and elute the RNA with 15-20 μl of DEPC-treated water (supplied with kit).** Make sure to add water directly onto column matrix. Let it sit at room temperature for 2 minutes. Centrifuge 1 min at maximum speed.

RNA may be eluted with a smaller (<15μl) volume of water to get higher RNA concentration. While reduced elution volume decrease total RNA yield. The total yield will be 20-30% less when the elution volume is less than 10μl.

D. Extraction of RNA from Fibrous tissues

Materials supplied by user

- absolute ethanol (96-100%)
- β-Mercaptoethanol
- Disposable latex gloves
- Water bath or heat block preset at 55°C
- Proteinase K (20mg/ml)

Note: *Equilibrate samples and TRK Lysis buffer to room temperature before start. All steps must be carried out at room temperature. Work quickly, but carefully. Depending on the process of the fixation protocol, storage condition, staining protocol, and the age of the sample, RNA can be highly degraded into small fragments less than 300nt. Since Total RNA isolation protocol will remove most fragments less than 200nt. This could lead to overall lose of RNA if the sample is highly degraded.*

1. **Collect the sample into a 1.5 ml centrifuge tube contains 200 μl TRK Lysis Buffer**. Remember to add 20 μl of 2-mercaptoethanol per 1 mL of TRK Lysis Buffer before use.

2. **Adjust the sample volume to 200 μl with TRK Lysis Buffer**. When processing small amounts of cells (≤5000 cells), add 3 μ1 of linear acrylamide and 1ul Carrier RNA to the lysate before homogenization.

3. Add 200 μl DEPC-Treated water into the sample. Then add 5 μl of Proteinase k (20mg/ml). Mix well by pipetting.

4. Incubate at 55°C for 10 minutes.

5. Centrifuge at 10,000 x g for 3 minutes at room temperature.

6. **Transfer the supernatant (about 400 μl) into a new tube.**

7. **Add 0.5 volume of absolute ethanol (96-100%).** Mix throughly by pipetting or vortexing.

8. **Place MicroElute® RNA column into a 2 ml collection tube (supplied with kit) and apply the mixture from step 7 (including any precipitate) to the column.** Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through and

Introduction

E.Z.N.A.™ MicroElute® Total RNA Kit provides a rapid and easy method for the isolation of up to 50 µg of total RNA from small amount of cultured eukaryotic cells, tissues such as laser dissected samples (LDS) or fine needle aspirates (FNA). Normally, up to 5 x 10^5 eukaryotic cells or 5 mg tissue (amounts depend on the tissue used) can be used in a single experiment. The kit allows single or multiple, simultaneous processing of samples in less than 30 min. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or LiCl, are eliminated.

RNA purified using the E.Z.N.A.™ MicroElute® Total RNA method is ready for applications such as RT-PCR*, Northern blotting, poly A^+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

Principle

The E.Z.N.A.™ MicroElute® Total RNA Kits combine the reversible binding properties of HiBind® matrix, a new silica-based material with the speed of mini-column spin technology. A specifically formulated high salt buffer system allows RNA molecules greater than 200 bases to bind to the matrix. Cells or tissues are first lysed under denaturing conditions that practically inactivate RNases. After add the ethanol, samples are then applied to the HiBind® MicroElute ® columns to which total RNA binds, while cellular debris and other contaminants are effectively washed away. High quality RNA is finally eluted in DEPC-treated sterile water.

Storage and Stability

E.Z.N.A.™ MicroElute ® Total RNA Kits should be stored at room temperature. During shipment crystals may form in the MRC Lysis Buffer. Warm to 37°C to dissolve. All E.Z.N.A.™ Total RNA Kit components are guaranteed for at least 24 months from the date of purchase when stored at 22-25°C.

DNA without DNase treatment. However, no RNA extraction procedure can completely remove genomic DNA. For sensitive work (such as RT-PCR or differential display) we suggest that you perform on-column DNase I digestion (OBI cat# E1091) or treat the eluted RNA with RNase-free DNase. Also for RT-PCR, use intron-spanning primers that allow easy identification of DNA-contamination.

Quantitation and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm in a spectrophotometer. 1 O.D. unit measured at 260 nm corresponds to 40 µg of RNA per mL. DEPC-water is slightly acidic and can dramatically lower absorbance values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometric analysis. The ratio of A_{260}/A_{280} of pure nucleic acids is 2.0, while for pure protein it is approximately 0.6. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. (Phenol has an absorbance maximum at 275 nm and can interfere with absorbance readings of DNA or RNA. However, the E.Z.N.A.® Total RNA Kit eliminates the use of phenol and avoids this problem.) Store RNA samples at -70°C in water. Under such conditions RNA prepared with the E.Z.N.A.® system is stable for more than a year.

RNA Quality

It is highly recommended that RNA quality be determined prior to all analyses. The quality of RNA can best be assessed by denaturing agarose gel electrophoresis and ethidium bromide staining. Two sharp bands should appear on the gel. These are the 28S and 18S (23S and 16S for bacteria) ribosomal RNA bands. If these band smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling, or storage. Although RNA molecules less than 200 bases in length do not efficiently bind the HiBind matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Trouble Shooting Guide

Problem	Cause	Suggestion

Disruption and Homogenization of Samples

For all RNA isolation process, it is absolutely critical to disrupt and homogenize the sample. Laser dissected samples (LDS) and fine needle aspirates (FNA) can be simultaneously disrupted and homogenized by vortexing. For other samples, select one of the following methods for disruption and homogenization.

A. Disruption with Liquid Nitrogen

Wear gloves and take great care when working with liquid nitrogen. Excise tissue and promptly freeze in a small volume of liquid nitrogen. Grind tissue with a ceramic mortar and pestle under approximately 10 mL of liquid nitrogen and pour the suspension into a pre-cooled 15 mL polypropylene tube. Unless the tube is pre-cooled (in liquid nitrogen), the suspension will boil vigorously possibly causing loss of tissue. When the liquid nitrogen has completely evaporated, add TRK Lysis Buffer and continue with the procedure as outlined below.

B. Homogenization Using Homogenizer column or fine needle:

After interrupt tissue, lysate can be homogenized with Omega Homogenizer Spin Column (Product # HCR-03). The lysate is loaded onto Omega Homogenizer Spin Column in a 2 mL collection tube. Spin two minutes at a maximum speed in a microcentrifuge and the homogenized lysate collected. Use the Omega Homogenizer Spin Column is fast and efficient way to homogenize the lysate without cross contamination of samples. The alternated way to homogenize the lysate is to use the syringe and needle. High molecular-weight DNA can be sheared with by passing the lysate through a narrow needle (19-21 gauge) for 5-10 times.

C. Disruption and Homogenization with Rotor-Stator

Rotor-Stator is the most preferred method for disruption and homogenizing tissue samples if required equipments are available. Rotor-stator homogenizers effectively homogenize and homogenize most tissues in the present of TRK Lysis Buffer . The process usually takes less than a minute depending on the tissue. Many Rotor-stator homogenizers operate with differently sized probes or generators that allow processing of small volumes in microfuge tubes.

Such homogenizers are available from:

- Tekmar Inc., Cincinnati, OH (Tissuemizers®)
- BIOSPEC Products, Bartlesville, OK (Tissue-Tearor™)
- Craven Laboratories, Austin, TX
- OMNI International Inc. Waterbury, CT

D. Disruption and Homogenization with Beads Mills

Tissue sample can also be effectively disrupted and homogenized by rapid agitation in the presence of beads and lysis buffer. Tissue samples are disrupted and simultaneously homogenized with the sheared and crushed action of the beads as they collide with cells.

This protocol is designed to recovery RNA from enzymatic reactions such as DNase I digestion, In vitro transcription, etc.

1. **Measure the volume of sample and adjust the sample volume to 100µl with DEPC-Water and proceed to step 2.**

2. **Add 350ul TRK Lysis buffer and mix by vortexing at maximum speed for 15 seconds. When process small amount of RNA (≤2 µg). Add 3 µl of Linear Acrylamide and 1ul Carrier RNA to the mixture.**
   Note: Remember to add 20 µl of 2-mercaptoethanol per 1 ml of TRK Lysis Buffer before use.

3. **Add 250ul absolute ethanol (96-100%, room temperature) to the sample and mix thoroughly by vortexing.**

4. Apply sample from step 3 to HiBind® MicroElute RNA column inserted in a 2 ml collection tube (supplied). The maximum capacity of the spin cartridge is 700 µl. (Larger volumes can be loaded successively.) Centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow-through and re-use the collection tube.

5. **Place column in the same 2 ml collection tube from step 4 and add 500 µl RWB Wash Buffer diluted with absolute ethanol.** Centrifuge and discard flow-through.
   Note: RWB Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

6. **Place column back into the same collection tube from step 5, and add another 500 µl RWB Wash Buffer diluted with absolute ethanol.** Centrifuge and discard the flow-through and collection tube.

7. Place the column into a new 2 ml collection tube (supplied). Centrifuge the column at full speed (≥13,000 x g) for 2 minutes. Discard the flow-through and the collection tube.

8. **Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and pipet 15-30 µl of DEPC-treated water (supplied with kit) into the column.** Make sure to add water directly onto center of column matrix. Incubate at room temperature for 2 min. Centrifuge for 1 min at maximum speed. A second elution may be necessary if the expected yield of RNA >20 µg.

F. On-membrane DNase I Digestion Protocol

Centrifuge and discard flow-through.

9. **Then with the collection tube empty, centrifuge the empty column for 2 min at full speed (≥13,000 x g) to completely dry the HiBind® matrix.**

10. **Elution of RNA. Transfer the column to a clean 1.5 mL centrifuge tube (not supplied with kit) and elute the RNA with 15-20 μl of DEPC-treated water (supplied with kit).** Make sure to add water directly onto center of membrane. Let it sit at room temperature for 2 minutes. Centrifuge for 1 min at full speed.

    RNA may be eluted with a smaller (<15μl) volume of water to get higher RNA concentration. While reduced elution volume decrease total RNA yield. The total yield will be 20-30% less when the elution volume is less than 10μl.

B. Extraction of RNA from Micro-Dissected Formalin-Fixed tissues

(skeletal muscle, heart and aorta tissue, et al.)

Materials supplied by user:

- 96-100% ethanol
- β-Mercaptoethanol
- RNase-free filter pipette tips
- Disposable latex gloves
- RNase-Free 1.5 mL tubes
- Water bath or heat block preset at 55°C
- Proteinase K (20mg/ml)

Note: Equilibrate samples and TRK Lysis Buffer to room temperature before start. All steps must be carried out at room temperature. Work quickly, but carefully.

1. Excise tissue from animal or from storage.

2. Weight up to ≤5 mg tissue and immediately place it into a 1.5 ml microcentrifuge tube for disruption and homogenization.

3. **Add 200 μl TRK Lysis Buffer and disrupt tissue and homogenize lysate in TRK Lysis buffer by using methods described on page 4.** Remember to add 20 μl of 2-mercaptoethanol per 1 mL of TRK Lysis Buffer before use.

    Note: Incomplete homogenization will cause clogging of the spin column and lead to significantly lower yield. Generally, disruption and homogenization by using mortar and pestle or needle and syringe can generate lower yield. It is recommenced to use a rotor stator homogenizer or beads milling methods for animal tissues.

4. **Pipet 200 μl RNase-Free water to each homogenate. The add 5 μl Proteinase k (20 mg/ml) and** mix through by pipetting or vortexing.

5. **Incubate at 55 °C for 10 minutes.**

6. **Centrifuge at ≥13,000 x g for 3 minutes at room temperature.**

7. **Transfer the supernatant (about 600 μl) to a new 1.5ml centrifuge tube and**

collection tube.

9. **Place column in a clean 2ml collection tube, and add 400 μl of RWC Wash Bufffer.** Centrifuge at 10,000 x g for 30 seconds. Discard flow-through and re-use the collection tube.

10. ***DNase I Digestion (Optional): This is point to start On-membrane DNase I digestion. (See detail procedure on page 14)***.

11. **Place column in the same 2 ml collection tube from step 9 or 10 and add 500 μl RWB Wash Buffer diluted with ethanol.** Centrifuge at 10, 000 x g for 30 seconds. Discard the flow-through and re-use the collection tube.
Note: RWB Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for instructions

12. **Wash column with a second 500 μl of RWB Wash Buffer as in step 11.** Centrifuge for 30 seconds at 10,000 x g and discard flow-through. **Then with the collection tube empty, centrifuge the column for 2 min at full speed (≥ 13,000 x g) to completely dry the HiBind® matrix.**

13. **Elution of RNA. Transfer the column to a clean 1.5 mL microfuge tube (not supplied with kit) and elute the RNA with 15-20 μl of DEPC-treated water (supplied with kit).** Make sure to add water directly onto column matrix. Let it sit at room temperature for 2 minutes. Centrifuge 1 min at maximum speed.

    RNA may be eluted with a smaller (<15μl) volume of water to get higher RNA concentration. While reduced elution volume decrease total RNA yield. The total yield will be 20-30% less when the elution volume is less than 10μl.

C. RNA Isolation From Animal tissue or cell culture

This method is designed for most animal tissues and culture cells. For RNA isolation from fibrous tissue, follow the specialized protocol on page 11. For laser dissected samples, please follow the protocol on page 5. All centrifugation step must be carried out at room temperature.

Materials supplied by user:

- 96-100% ethanol
- β-Mercaptoethanol
- RNase-free filter pipette tips
- Microcentrifuge capable of 12,000 x g

1. **Determine the starting amount of sample. Do not use more than 5 x 10^5 cells or 5 mg tissue.**

2. **Lyse cells (< 5x 10^5) or tissues (< 5mg) with 350 μl of TRK Lysis Buffer.** Remember to add 20 μl of 2-mercaptoethanol per 1 mL of TRK Buffer before use.

3. **Disrupt the tissue or cells and Homogenize the lysate in TRK Lysis Buffer according to one of the methods on page 4.** When processing small amounts of cells (≤ 5000 cells). Add 3μl of linear acrylamide and 1ul Carrier RNA to the lysate before homogenization.

4. Centrifuge at 13,000 x g for 3 minutes at room temperature when processing animal tissue.

5. **Transfer the supernatant to a new 1.5 ml centrifuge tube and add equal volume of 70% ethanol to the lysate.** Mix thoroughly by vortexing or pipetting.

6. **Apply the mixture from step 5 onto HiBind® MicroElute® RNA column pre-inserted in a 2 mL collection tube.** The maximum capacity of the spin cartridge is 750 μl. A precipitate may form on addition of ethanol in step 5. Vortex and add the entire mixture to the column. With the spin column inside a 2ml collection tube (supplied with kit), centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow-through and the collection tube.

7. **Place column in a new 2 ml collection tube (supplied), and add 400 μl RWC Wash Bufffer.** Centrifuge and discard flow-through. Reuse the collection tube in step 8 or step 9. If on-membrane DNase I digestion is desired, proceed step 8, otherwise go to step 9.