HB3600

Allspin™

For total DNA & RNA isolation from tissues and cultured cells

TOTAL DNA/RNA PURIFICATION HANDBOOK

GeneAll

Customer & Technical Support

Should you have any further questions, do not hesitate to contact us.

We appreciate your comments and advice.

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www.geneall.com

www.geneall.co.kr

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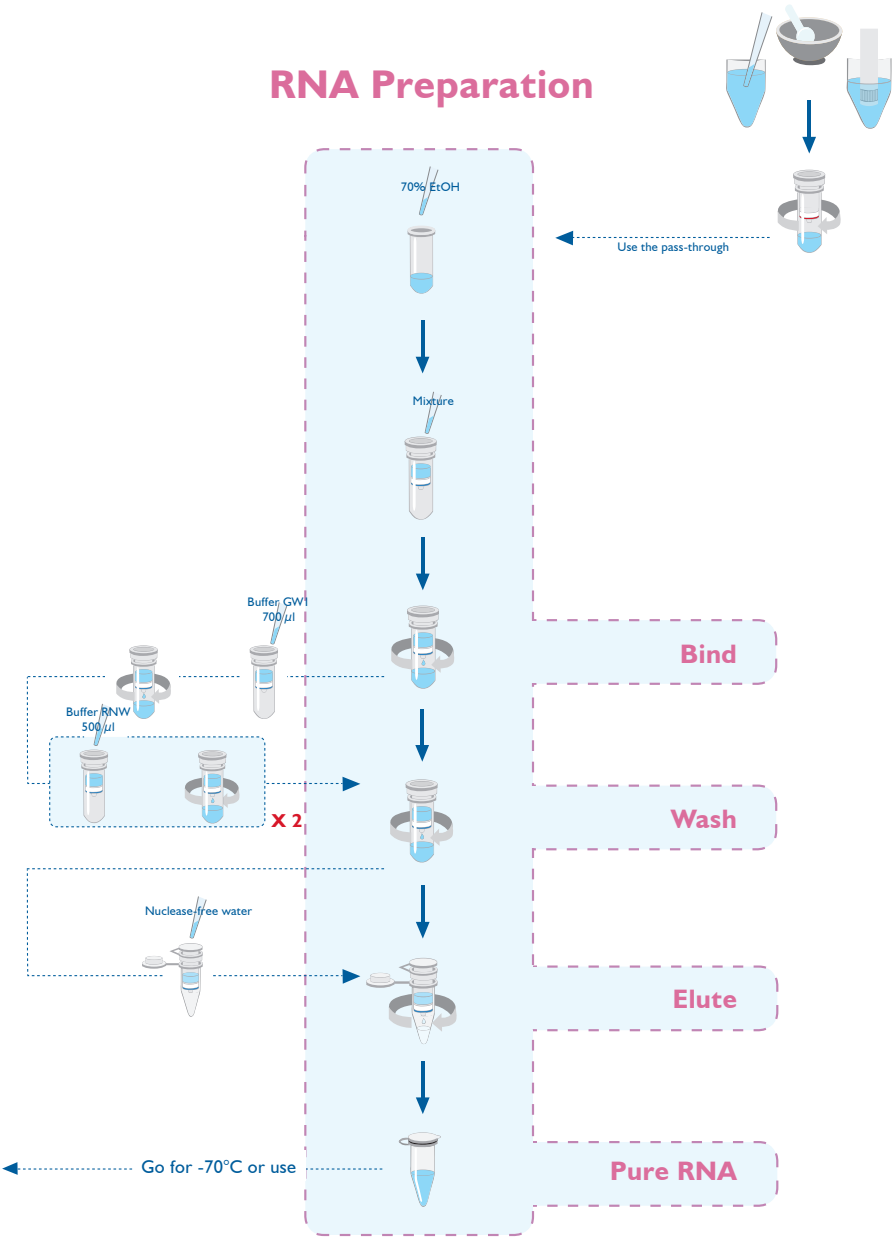
This protocol handbook is included in :

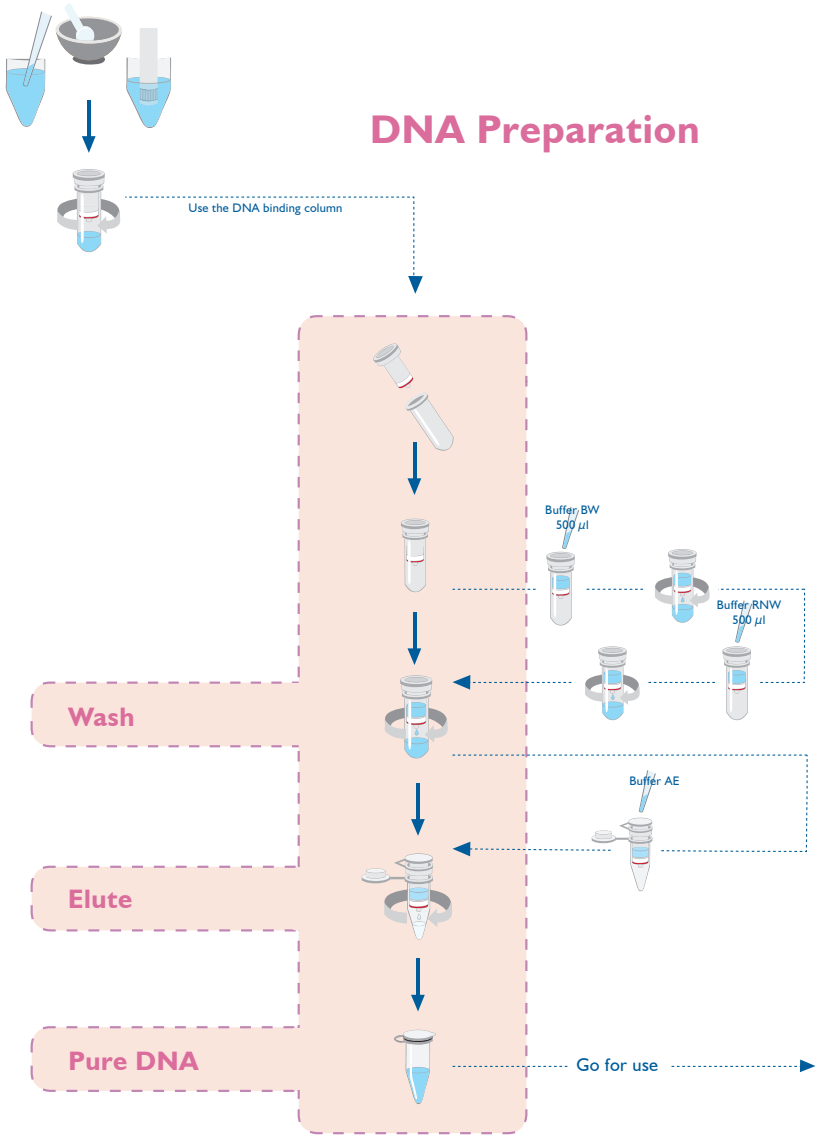
GeneAll® Allspin™ (306-150)

Visit www.geneall.com or www.geneall.co.kr for FAQ, Q&A and more information.

Brief Protocol

RNA Preparation




DNA Preparation
Use the DNA binding column
Buffer BW
500 µl
Buffer RNW
500 µl
Buffer AE
Wash
Elute
Pure DNA
Go for use

INDEX

Kit Contents

Cat. No.	306-150	Storage
Components	Quantity	
Buffer CTL	45 ml	Room temperature (15~25°C)
Buffer GW1	45 ml	
Buffer BW (concentrate) *	16 ml	
Buffer RNW (concentrate) * †	12 ml x 2	
Buffer AE **	15 ml	
Nuclease-free water	15 ml	
Mini column type B (with collection tube)	50	
Column type W (mini) (with collection tube)	50	
2.0 ml collection tube	50	
1.5 ml microcentrifuge tube	100	

** Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and RNW as indicated on the bottle.*

† Contains sodium azide as a preservative

*** 10 mM TrisCl, pH 9.0, 0.5 mM EDTA*

Materials Not Provided

Reagents

- Tissue storage buffer to protect RNA from RNase
- Absolute ethanol, ACS grade or better

Disposable materials

- RNase-free pipet tips
- Disposable gloves

Equipments

- Equipment for disrupting sample
- Microcentrifuge, Vortex mixer
- Suitable protector (ex; lab coat, goggles, etc)

Product Specifications

Allspin™ total DNA / RNA purification kit		
Specification	**Column type B for DNA**	**Column type W for RNA**
Type	Spin / mini	Spin / mini
Maximum amount of starting samples	30 mg / prep or ~1 x 10^7 cells / prep	30 mg / prep or ~1 x 10^7 cells / prep
Preparation time	≥30 min	≥30 min
Maximum loading volume of mini column	750 μl	750 μl
Minimum elution volume	50 μl	30 μl

Quality Control

All components in GeneAll® Allspin™ are manufactured in strictly clean conditions, and its degree of cleanness is monitored periodically. Quality control is carried out thoroughly from lot to lot, and only the qualified kits are approved to be delivered.

Storage Conditions

All components of GeneAll® Allspin™ should be stored at room temperature (15~25°C). It should be protected from exposure to direct sunlight.
During shipment or storage under cool ambient condition, a precipitate can be formed in Buffer CTL, GW1, and BW. In such a case, heat the bottle to 50°C to dissolve completely. Using precipitated buffers will lead to poor DNA recovery. GeneAll® Allspin™ is guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in the Allspin™ total DNA / RNA purificaion kit contain irritants which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken when handling such materials. Always wear gloves and eye protection, and follow standard safety precautions.

Buffer CTL, GW1, and BW contain chaotropic agents, which can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Product Disclaimer

GeneAll® Allspin™ is for research use only, not for use in diagnostic procedure.

Preventing RNase Contamination

RNase can be introduced accidentally during RNA purification. Wear disposable gloves always, because skin often contains bacteria and molds that can be a source of RNase contamination. Use sterile, disposable plastic wares and automatic pipettes to prevent cross-contamination of RNase from shared equipment.

Sample Amount and Average Yield

Amount of starting material	Sample type	Average yield of genomic DNA	Average yield of total RNA
Cultured cells (≒ 1 x 10^6)	CHO	~7 μg	~15 μg
	RAW264.7	~10 μg	~20 μg
Tissue (rat) (10 mg / prep)	Liver	~25 μg	~60 μg
	Kidney	~25 μg	~30 μg
	Brain	~12 μg	~10 μg
	Heart	~10 μg	~9 μg
	Spleen	~70 μg	~80 μg

The yield of genomic DNA and total RNA may vary depending on the type of tissue or cells from which it is obtained.

Product Description

Allspin™ total DNA / RNA purification kit provides a convenient method for the isolation of total DNA and total RNA simultaneously from a single sample of tissue or cultured cells. It utilizes the optimized buffer system and the advanced silica-binding technology to purify nucleic acid sufficiently pure for many applications, instead of conventional alcohol precipitation and phenol / chloroform extraction.

DNA and RNA are purified separately from a same sample but successive procedure using Column Type B and W respectively. Alternatively, both DNA and RNA can be co-purified into a single tube by the modified procedure at appendix 1. Whole procedure can be performed in just 30 minutes and the length of obtained DNA is up to 50 kb (average is 30 kb) and that of RNA is longer than 200 nucleotides.

To obtain pure DNA, samples are homogenized in Buffer CTL, containing guanidine salt, which rapidly lyses cells and inactivates nucleases and then the lysate is applied into Column type B. During centrifugation, DNA is bound specifically to column membrane and RNA is passed through it and goes into the collection tube. The membrane is washed by a series of washing buffers and bound DNA is eluted by Buffer AE.

For purification of pure RNA, the passed-through which is obtained from fraction of DNA purification procedure is mixed with ethanol and this mixture is loaded into column W. RNA is bound specifically during centrifugation. After washed by Buffer GW1 and RNW, pure RNA is eluted by Nuclease-free water.

Purified DNA and RNA are fully suitable for the isolation of Poly A^+ RNA, southern and northern blotting, dot blotting, in vitro transcription, cloning, RT-PCR and other analytical procedures.

Allspin™ total DNA / RNA purificaion kit

PROTOCOL
for cultured animal cells

Before experiment

* *Before first use, add absolute ethanol (ACS grade or better) to Buffer BW, Buffer RNW as indicated on the bottle.*
* *Prepare 70% ethanol*
* *All centrifugation should be carried out at room temperature in a microcentrifuge.*

1. Harvest cell samples in a 1.5 ml microcentrifuge tube.

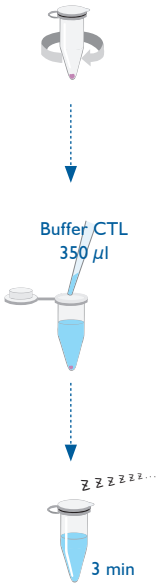
Cells grown in monolayer

Harvest 5 x 10^6 cells carefully using scraper. And pellet cells by centrifugation at low speed (below 3000 rpm) for 5 min, then discard the culture medium.

Cells grown in suspension

Pellet 5 x 10^6 cells by centrifugation at low speed (below 3000 rpm) for 5 min, then discard the culture medium.

* Do not wash cells before lysing with Buffer CTL as this may contribute to mRNA degradation.



2. Add 350 µl of Buffer CTL to the tube and lyse the sample by pipetting or homogenizing.

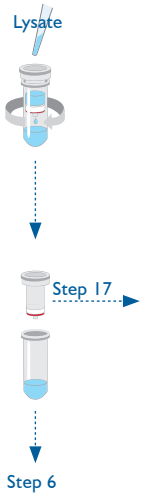
Lyse the 5 x 10^6 cells in 350 µl Buffer CTL using pipetting. An insufficient lysis may result in low RNA recovery rate or mini column clogging.

3. Incubate the lysate for 3 min at room temperature.

This step allows nucleoprotein complexes to completely dissociate.

4. **Transfer the lysate to a Column Type B (red ring). Centrifuge at ≥10,000 x g for 30 sec at room temperature.**
Maximun volume is 750 μl and DNA is bound to membrane through this step.

5. **Transfer the mini column to a new 2.0 ml collection tube (provided), and store at room temperature. Use the pass-through for total RNA purification.**
Make sure that no lysate remains in the mini column after centrifugation. If the residual lysate has remained, centrifuge again at higher speed until all of the solution has passed through.



Cell

Go on to step 6 for total RNA purification from cell samples.

Go on to step 17 for genomic DNA purification from cell samples.

Total RNA purification (Blue ring column step)

6. **Add 1 volume (usually 350 μl) of 70% ethanol (not provided) to the collection tube including the pass-through (step 5), and mix well by pipetting.**
Do not centrifuge.

7. **Transfer the mixture (approximately 700 μl) including any precipitate to a Column Type W (blue ring).**

8. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

9. **Add 700 µl of Buffer GW1 to the mini column.**

10. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

11. **Add 500 µl of Buffer RNW to the mini column.**

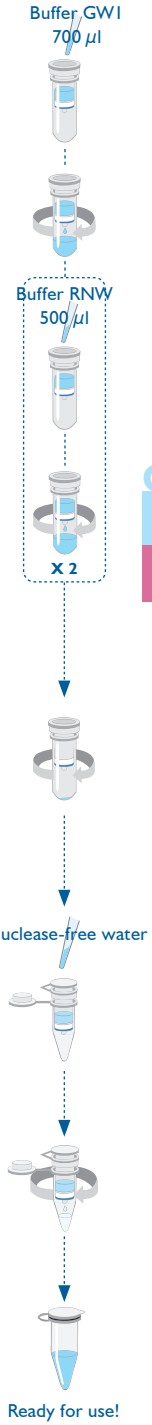
12. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

13. **Repeat the step 11 and 12.**

14. **Centrifuge at ≥10,000 x g for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube (provided).**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of Buffer RNW.

15. **Add 50 µl of Nuclease-free water to the center of the membrane in the mini column.**
    According to the expected yield, the volume of eluent can be adjusted.

16. **Centrifuge at ≥10,000 x g for 1 min at room temperature.**
    Purified RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.



Genomic DNA purification (Red ring column step)

17. **Add 500 µl of Buffer BW to the mini column.**

18. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

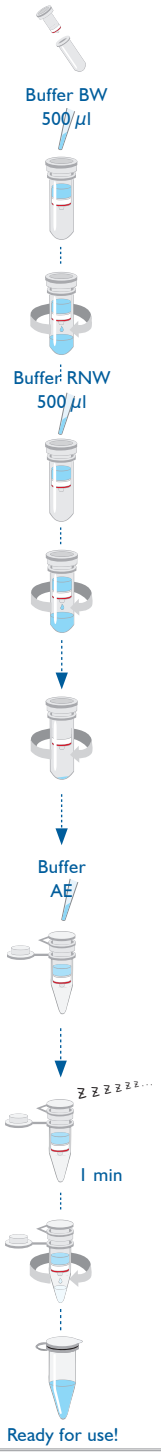
19. **Add 500 µl of Buffer RNW to the mini column.**

20. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

21. **Centrifuge at ≥10,000 x g for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube (provided).**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of Buffer RNW.

22. **Add 50 µl of Buffer AE to the center of the membrane in the mini column. Let it stand for 1 min.**
    According to the expected yield, the volume of eluent can be adjusted.

23. **Centrifuge at ≥10,000 x g for 1 min at room temperature.**
    For long-term storage, eluting in Buffer AE is recommended. But, EDTA included in Buffer AE can inhibit some delicate enzymatic reaction, so you can avoid such latent problems by using distilled water (>pH 7.0) or Tris-Cl (>pH 8.5). When using water for elution, make sure the pH of water is higher than 7.0.



Allspin™ total DNA / RNA purificaion kit

PROTOCOL
for animal tissues

Before experiment

* *Before first use, add absolute ethanol (ACS grade or better) to Buffer BW, Buffer RNW as indicated on the bottle.*
* *Prepare 70% ethanol*
* *All centrifugation should be carried out at room temperature in a microcentrifuge.*

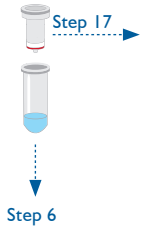
1. **Harvest tissue samples in a 1.5 ml microcentrifuge tube.**
   Harvest ~20 mg tissue samples in a 1.5 ml microcentrifuge tube. The recommended method for sample handling is to put directly removed fresh tissue into tissue storage buffer or to freeze the tissue rapidly in liquid nitrogen.

2. **Add 350 µl of Buffer CTL to the tube and disrupt and homogenize the sample by homogenization.**
   Before starting, add ß-mercaptoethanol (10 µl per 1 ml) to Buffer CTL.
   Homogenize ~20 mg of tissue samples in 350 µl Buffer CTL using homogenizer (rotor-stator homogenizer, mortar and pestle, or bead-beater). Thoroughly disrupt the tissue in Buffer CTL and lyse the samples completely. Not clarified sample may cause clogging of the mini column in subsequent steps.

3. **Incubate the lysate for 10 min at room temperature.**
   This step allows nucleoprotein complexes to completely dissociate and reduces the generated foam.

4. **Centrifuge at maximum speed for 3 min at room temperature and carefully transfer the supernatant to a Column Type B (red ring).**
   This step can help avoid clogging of the mini column caused by not clarified insoluble particles.
   Maximun volume is 700 μl and DNA is bound to membrane through this step.

5. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Transfer the mini column to a new 2.0 ml collection tube (provided), and store at room temperature. Use the pass-through for total RNA purification.**
   Make sure that no lysate remains in the mini column after centrifugation. If the residual lysate has remained, centrifuge again at higher speed until all of the solution has passed through.



Go on to step 6 for total RNA purification from tissue sample.

Go on to step 17 for genomic DNA purification from tissue sample.

Total RNA purification (Blue ring column step)

6. **Add 1 volume (usually 350 μl) of 70% ethanol to the collection tube including the pass-through (step 5), and mix well by pipetting. Do not centrifuge.**

7. **Transfer the mixture (approximately 700 μl) including any precipitate to a Column Type W (blue ring).**

8. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

9. **Add 700 µl of Buffer GW1 to the mini column.**

10. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

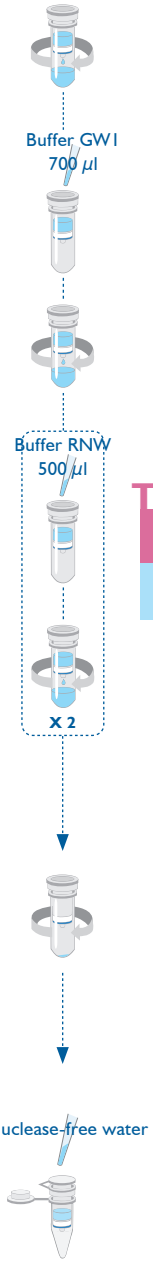
11. **Add 500 µl of Buffer RNW to the mini column.**

12. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

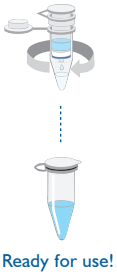
13. **Repeat the step 11 and 12.**

14. **Centrifuge at ≥10,000 x g for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube (provided).**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of Buffer RNW.

15. **Add 50 µl of Nuclease-free water to the center of the membrane in the mini column.**
    According to the expected yield, the volume of eluent can be adjusted.



16. **Centrifuge at ≥10,000 x g for 1 min at room temperature.**
Purified RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.
In tissue samples, occasionally genomic DNA contamination is happened in the RNA eluate because of lots of sample amount. In this case, reduce the starting sample amounts or use a DNase I following the manufacture's instruction manual.



Genomic DNA purification (Red ring column step)

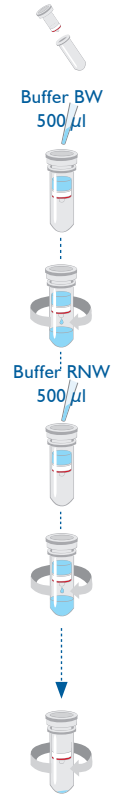
17. **Add 500 µl of Buffer BW to the mini column.**

18. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

19. **Add 500 µl of Buffer RNW to the mini column.**

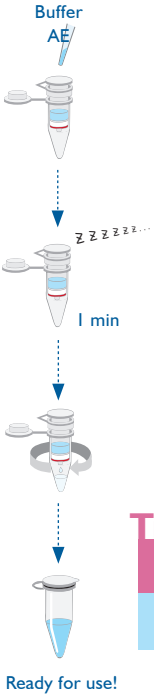
20. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

21. **Centrifuge at ≥10,000 x g for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube (provided).**
Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of Buffer RNW.



22. **Add 100 µl of Buffer AE to the center of the membrane in the mini column. Let it stand for 1 min.**
According to the expected yield, the volume of eluent can be adjusted.

23. **Centrifuge at ≥10,000 x g for 1 min at room temperature.**
For long-term storage, eluting in Buffer AE is recommended. But, EDTA included in Buffer AE can inhibit some delicate enzymatic reaction, so you can avoid such latent problems by using distilled water (>pH 7.0) or Tris-Cl (>pH 8.5). When using water for elution, make sure the pH of water is higher than 7.0.



Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield	**Insufficient homogenization of the sample with Buffer CTL**	Be sure to incubate after homogenization. Confirm the completely homogenized sample in Buffer CTL.
	Too much starting sample	Reduce the amount of starting sample. Especially for tissue sample, use the correct amount of starting sample.
	Poor quality of starting material	Process the sample immediately after harvest from animal. To process later, freeze the tissue rapidly in liquid nitrogen.
	Residual culture media in the sample	Remaining culture media affect lysis and binding condition of Buffer CTL.
Column clogging	**Insufficient homogenization of the sample with Buffer CTL**	Be sure to incubate after homogenization. Confirm the completely lysed sample in Buffer CTL.
	Too much starting sample	Reduce the amount of starting sample. Especially for tissue sample, use the correct amount of starting sample.
Low A_{260} / A_{280}	**Insufficient homogenization of the sample with Buffer CTL**	Be sure to incubate after homogenization. Confirm the completely lysed sample in Buffer CTL.
High A_{260} / A_{280} in DNA eluate	**RNA contamination**	RNA may inhibit some downstream enzymatic reactions. If RNA-free DNA is required, RNase treatment should be performed following the manufacture's protocol.
	Water used instead of Buffer AE to dilute DNA or to make blank for A_{260} / A_{230} measurement	Use Buffer AE to dilute extracted nucleic acid and to make blank before measure purity, If Buffer AE has been used for elution.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
DNA contamination in RNA eluate	**Too much starting sample**	Reduce the amount of starting sample. Especially for tissue sample, use the correct amount of starting sample.
	No treatment Buffer GW1	Follow the Allspin™ total DNA / RNA purification kit manual.
	High DNA mass in sample	Especially, some tissue samples have high DNA content. In this case, some DNA will pass the Column Type B. And then bind to the next RNA binding step at Column Type W with RNA. Reduce the amount of starting sample or perform DNase digestion at the RNA eluate.
RNA contamination in DNA eluate	**Too much manipulated sample before process**	Reduce the amount of starting sample. Especially for tissue sample, use the correct amount of starting sample.
RNA degradation	**Too much manipulated sample before process**	Process the sample immediately after harvest from animal. For cultured cell, minimize washing steps in cell harvest.
	Improper storage of RNA	Store isolated RNA at -70°C, Do not store at -20°C.
	Use of not RNase-free reagent or disposable products	Make sure to use RNase-free products only.
DNA degradation	**Too old or mis-stored starting sample**	Too old or mis-stored sample often yield degraded DNA. Use fresh sample.
	Vigorous homogenization	Vigorous handling after addition of Buffer CTL can lead to irreversible denaturation of genomic DNA. Minimize the homogenization time and gently homogenize the sample.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Eluate does not perform well in downstream application	**Residual ethanol in eluate**	To remove any residual ethanol included in Buffer RNW from mini column membrane, centrifuge again for complete removal of ethanol.
	Use of Buffer BW and RNW in the wrong order	Ensure that Buffer BW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with Buffer RNW.

APPENDIX I. Copurification of total DNA and RNA in a single tube

Allspin™ total DNA / RNA purificaion kit is convenient kit for isolation of genomic DNA and total RNA from tissues or cultured cells. Allspin™ total DNA / RNA purificaion kit is designed to purify DNA and RNA separately, but, DNA and RNA can be purified simultaneously in a single tube using the modified method. After lysis, the lysate is mixed with ethanol then apply to Column Type B to bind DNA and RNA on the membrane. And then the impurities on the membrane are washed away by two different wash buffers. At last pure RNA and DNA are eluted by Nuclease-free water. The eluate should be treated with care because RNA is very sensitive to contamination.

Protocol for cultured cell samples.

1. **Harvest cell samples in a 1.5 ml microcentrifuge tube.**
   Cells grown in monolayer
   Harvest 5 x 10^6 cells carefully using scraper. And pellet cells by centrifugation at low speed (below 3000 rpm) for 5 min, then discard the culture medium.

   Cells grown in suspension
   Pellet 5 x 10^6 cells by centrifugation at low speed (below 3000 rpm) for 5 min, then discard the culture medium.
   * Do not wash cells before lysing with Buffer CTL as this may contribute to mRNA degradation.

2. **Add 350 μl of Buffer CTL to the tube and lyse the sample by pipetting or homogenizing.**
   Lyse the 5 x 10^6 cells in 350 μl Buffer CTL using pipetting. An insufficient lysis may result in low RNA recovery rate or column clogging.

3. **Incubate the lysate for 3 min at room temperature.**
   This step allows nucleoprotein complexes to completely dissociate.

4. **Add 1 volume (usually 350 μl) of absolute ethanol to the lysate, and mix well by pipetting or vortexing. Do not centrifuge.**

5. **Transfer the mixture (approximately 700 μl) including any precipitate to a Column Type B (red ring).**

6. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

7. **Add 500 μl of Buffer BW to the mini column.**

8. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

9. **Add 500 μl of Buffer RNW to the mini column.**

10. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

11. **Centrifuge at ≥10,000 x g for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube (provided).**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of Buffer RNW.

12. **Add 100 μl of Nuclease-free water to the center of the membrane in the mini column. Let it stand for 1 min.**
    According to the expected yield, the volume of eluent can be adjusted.

13. **Centrifuge at ≥10,000 x g for 1 min at room temperature.**

Protocol for tissue samples.

1. **Harvest tissue samples in a 1.5 ml microcentrifuge tube.**
   Harvest ~20 mg tissue samples in a 1.5 ml microcentrifuge tube. The recommended method for sample handling is to put directly removed fresh tissue into tissue storage buffer or to freeze the tissue rapidly in liquid nitrogen.

2. **Add 350 μl of Buffer CTL to the tube and disrupt and homogenize the sample by homogenization.**
   Before starting, add ß-mercaptoethanol (10 μl per 1 ml) to Buffer CTL.
   Homogenize ~20 mg of tissue samples in 350 μl Buffer CTL using homogenizer (rotor-stator homogenizer, mortar and pestle, or bead-beater). Thoroughly disrupt the tissue in Buffer CTL and lyse the samples completely. Not clarified sample may cause clogging of the mini column in subsequent steps.

3. **Incubate the lysate for 10 min at room temperature.**
   This step allows nucleoprotein complexes to completely dissociate and reduces the generated foam.

4. **Centrifuge at maximum speed for 3 min at room temperature and carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube (not provided).**
   This step can help avoid clogging of the mini column caused by not clarified insoluble particles.
   Maximun volume is 700 μl and DNA is bound to membrane through this step.

5. **Add 1 volume (usually 350 μl) of absolute ethanol to the lysate, and mix well by pipetting or vortexing. Do not centrifuge.**

6. **Transfer the mixture (approximately 700 μl) including any precipitate to a Column Type B (red ring).**

7. **Centrifuge at ≥ 10,000 x g for 30 sec at room temperature.**
   Discard the pass-through and reinsert the mini column back into the collection tube.

8. **Add 500 μl of Buffer BW to the mini column.**

9. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Add 500 μl of Buffer RNW to the mini column.**

11. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

12. **Centrifuge at ≥10,000 x g for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube (provided).**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of Buffer RNW.

13. **Add 100 μl of Nuclease-free water to the center of the membrane in the mini column. Let it stand for 1 min.**
    According to the expected yield, the volume of eluent can be adjusted.

14. **Centrifuge at ≥10,000 x g for 1 min at room temperature.**

APPENDIX 2. Confirmation of RNA yield and purity by UV absorbance

Concentration of RNA

The concentration of RNA can be determined by the absorbance at 260 nm using spectrophotometer. For the convenient measurement, we recommend using the NanoDrop® which can reduce your RNA sample and time. If not, you need to dilute the RNA samples to measure the concentration through traditional spectrophotometer. The value of A_{260} should be between 0.15 and 1.00. Be sure to calibrate the spectrophotometer with the same solution used for dilution.
An absorbance of 1 at 260 nm is correspond to about 40 μg RNA / ml at a neutral pH. Therefore, the concentration of RNA was calculated by the formula shown below.

A_{260} X dilution factor X 40 = RNA μg / ml

Purity of RNA

To confirm the RNA purity, you should read the ratio of A_{260} / A_{280}. Pure RNA is in the range of 1.8~2.2.

APPENDIX 3. Formaldehyde agarose gel electrophoresis (Denaturing gel method)

A denaturing agarose gel is routinely used for the assessment of the quality of an RNA preparation. After preparation, RNA forms secondary structure via intramolecular base pairing. Therefore, it is very difficult to get the exact result of electrophoresis because of migrating inaccuracy. However, the denaturing gel denatures the secondary structure of RNA and makes an accurate migration. To confirm the RNA band, the gel should be transferred to a UV transilluminator after electrophoresis. Mainly, two RNA bands are shown. In case of animal sample, the 28S and 18S rRNA bands are confirmed on the gel. If they are intact, the RNA bands should be sharp and the intensity of upper band should be about twice that of the lower band.

Prepare the denaturing gel

1. Put 1 g agarose in 72 ml water and heat to dissolve thoroughly.
2. Cool to 60°C.
3. Add 10 ml of 10X MOPS buffer, 18 ml of 37% formaldehyde, and 1 μl of a 10 mg / ml ethidium bromide (EtBr).
4. Mix well then pour the gel into the gel tray and cool to solidify it.
5. Transfer the solidified gel from tray to tank, and add enough 1X MOPS running buffer to cover the gel.

Prepare the RNA sample

1. Make the mixture.
   ? μl RNA (up to 20 μg)
   2 μl 10X MOPS electrophoresis buffer
   4 μl formaldehyde
   10 μl formamide
2. Incubate the mixture for 15 minutes at 65°C.
3. Chill the sample for 5 minutes in ice.
4. Add 2 μl of 10X formaldehyde gel-loading dye to the mixture.
5. Load the mixture in a denaturing gel which is covered with a sufficient 1X MOPS electrophoresis buffer.
6. Run the gel and confirm the RNA band on transilluminator.
   Occasionally, gel destaining may be needed to increase the visibility of the bands of RNA in dH_2O for several hours.

Composition of buffers

- 10X MOPS buffer
  0.2 M MOPS
  20 mM sodium acetate
  10 mM EDTA
  pH to 7.0 with NaOH

- 10X formaldehyde gel-loading dye
  50% glycerol
  10 mM EDTA
  0.25% (w / v) bromophenol blue
  0.25% (w / v) xylene cyanol FF

*** Caution**

When working with these chemicals, always use gloves and eye protector to avoid contact with skin and cloth. Especially, formaldehyde and ethidium bromide (EtBr) should be handled in a fume hood.

Ordering Information

GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

GeneAll® *Exprep™* *for preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid SV	mini	50	101-150	spin / vacuum
		200	101-102	
	Midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

GeneAll® *Exfection™* *for preparation of transfection-grade plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid LE *(Low Endotoxin)*	mini	50	111-150	spin / vacuum
		200	111-102	
	Midi	26	111-226	spin / vacuum
		100	111-201	
Plasmid EF *(Endotoxin Free)*	Midi	20	121-220	spin
		100	121-201	

GeneAll® *Expin™* *for purification of fragment DNA*

Products	Scale	Size	Cat. No.	Type
Gel SV	mini	50	102-150	spin / vacuum
		200	102-102	
PCR SV	mini	50	103-150	spin / vacuum
		200	103-102	
CleanUp SV	mini	50	113-150	spin / vacuum
		200	113-102	
Combo GP	mini	50	112-150	spin / vacuum
		200	112-102	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Tissue SV	mini	100	104-101	spin / vacuum
		250	104-152	
	Midi	26	104-226	spin / vacuum
		100	104-201	
	MAXI	10	104-310	spin / vacuum
		26	104-326	
Tissue *plus!* SV	mini	100	109-101	spin / vacuum
		250	109-152	
	Midi	26	109-226	spin / vacuum
		100	109-201	
	MAXI	10	109-310	spin / vacuum
		26	109-326	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Blood SV	mini	100	105-101	spin / vacuum
		250	105-152	
	Midi	26	105-226	spin / vacuum
		100	105-201	
	MAXI	10	105-310	spin / vacuum
		26	105-326	
Cell SV	mini	100	106-101	spin / vacuum
		250	106-152	
	MAXI	10	106-310	spin / vacuum
		26	106-326	
Clinic SV	mini	100	108-101	spin / vacuum
		250	108-152	
	Midi	26	108-226	spin / vacuum
		100	108-201	
	MAXI	10	108-310	spin / vacuum
		26	108-326	
Genomic DNA micro		50	118-050	spin
Plant SV	mini	100	117-101	spin / vacuum
		250	117-152	
	Midi	26	117-226	spin / vacuum
		100	117-201	
	MAXI	10	117-310	spin / vacuum
		26	117-326	
Soil DNA mini	mini	50	114-150	spin
Stool DNA mini	mini	50	115-150	spin
Viral DNA / RNA	mini	50	128-150	spin
FFPE Tissue DNA	mini	50	138-150	spin
		250	138-152	

GeneAll® *GenEx™* *for isolation of total DNA without spin column*

Products	Scale	Size	Cat. No.	Type
GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
	Lx	100	220-301	solution
GenEx™ Cell	Sx	100	221-101	solution
		500	221-105	
	Lx	100	221-301	solution
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
	Lx	100	222-301	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *GenEx™* *for isolation of total DNA*				
GenEx™ Plant	Sx	100	227-101	solution
	Mx	100	227-201	
	Lx	100	227-301	
GenEx™ Plant *plus!*	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	
GeneAll® *DirEx™* series *for preperation of PCR-template without extraction*				
DirEx™		100	250-101	solution
DirEx™ *Fast*-Tissue		96 T	260-011	solution
DirEx™ *Fast*-Cultured cell		96 T	260-021	solution
DirEx™ *Fast*-Whole blood		96 T	260-031	solution
DirEx™ *Fast*-Blood stain		96 T	260-041	solution
DirEx™ *Fast*-Hair		96 T	260-051	solution
DirEx™ *Fast*-Buccal swab		96 T	260-061	solution
DirEx™ *Fast*-Cigarette		96 T	260-071	solution
GeneAll® RNA series *for preperation of total RNA*				
RiboEx™	mini	100	301-001	solution
		200	301-002	
Hybrid-R™	mini	100	305-101	spin
Hybrid-R™ Blood RNA	mini	50	315-150	spin
Hybrid-R™ miRNA	mini	50	325-150	spin
RiboEx™ LS	mini	100	302-001	solution
		200	302-002	
Riboclear™	mini	50	303-150	spin
Riboclear™ *plus!*	mini	50	313-150	spin
Ribospin™	mini	50	304-150	spin
Ribospin™ II	mini	50	314-150	spin
		300	314-103	
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin ™ vRD *plus!*	mini	50	312-150	spin
Ribospin ™ vRD II	mini	50	322-150	spin
Ribospin ™ Plant	mini	50	307-150	spin
Ribospin™ Seed / Fruit	mini	50	317-150	spin
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351- 001	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *AmpONE™* *for PCR amplification*				
Taq DNA polymerase		250 U	501-025	(2.5 U/㎕)
		500 U	501-050	
		1,000 U	501-100	
α-Taq DNA polymerase		250 U	502-025	(2.5 U/㎕)
		500 U	502-050	
		1,000 U	502-100	
α-Pfu DNA polymerase		250 U	504-025	(2.5 U/㎕)
		500 U	504-050	
		1,000 U	504-100	
Fast-Pfu DNA polymerase		250 U	505-025	(2.5 U/㎕)
		500 U	505-050	
		1,000 U	505-100	
Hotstart Taq DNA polymerase		250 U	531-025	(2.5 U/㎕)
		500 U	531-050	
		1,000 U	531-100	
Taq Premix	96 tubes	20 ㎕	521-200	lyophilized
		50 ㎕	521-500	
		20 ㎕	526-200	solution
		50 ㎕	526-500	
α-Taq Premix	96 tubes	20 ㎕	522-200	lyophilized
		50 ㎕	522-500	
		20 ㎕	527-200	solution
		50 ㎕	527-500	
HS-Taq Premix	96 tubes	20 ㎕	525-200	solution
		50 ㎕	525-500	
		20 ㎕	520-200	lyophilized
α-Pfu Premix	96 tubes	50 ㎕	523-500	solution
Taq Premix (w/o dye)	96 tubes	20 ㎕	524-200	lyophilized
dNTPs mix		500 ㎕	509-020	2.5 mM each
dNTPs set (set of dATP, dCTP, dGTP and dTTP)		1 ml x 4 tubes	509-040	100 mM

Products	Scale	Size	Cat. No.	Type

GeneAll® *AmpMaster™* *for PCR amplification*

Taq Master mix	0.5 ml x 2 tubes	541-010	solution
	0.5 ml x 10 tubes	541-050	solution
α-Taq Master mix	0.5 ml x 2 tubes	542-010	solution
	0.5 ml x 10 tubes	542-050	solution
HS-Taq Master mix	0.5 ml x 2 tubes	545-010	solution
	0.5 ml x 10 tubes	545-050	solution
α-Pfu Master mix	0.5 ml x 2 tubes	543-010	solution
	0.5 ml x 10 tubes	543-050	solution

GeneAll® *HyperScript™* *for Reverse Transcription*

Reverse Transcriptase	10,000 U	601-100	solution
RT Master mix	0.5 ml × 2 tubes	601-710	solution
RT Master mix with oligo $(dT)_{20}$	0.5 ml × 2 tubes	601-730	solution
RT Master mix with random hexamer	0.5 ml × 2 tubes	601-740	solution
RT Premix	96 tubes, 20 $\mu\ell$	601-602	solution
RT Premix with oligo $(dT)_{20}$	96 tubes, 20 $\mu\ell$	601-632	solution
RT Premix with random hexamer	96 tubes, 20 $\mu\ell$	601-642	solution
One-step RT-PCR Master mix	0.5 ml × 2 tubes	602-110	solution
One-step RT-PCR Premix	96 tubes, 20 $\mu\ell$	602-102	solution
First strand Synthesis Kit	50 reaction	605-005	solution
ZymAll™ RNase Inhibitor	10,000 U	605-010	solution
ZymAll™ RNase Inhibitor	4,000 U	605-004	solution

GeneAll® RealAmp™ *for qPCR amplification*

SYBR qPCR Master mix (2X, Low ROX)	200 rxn 20 $\mu\ell$	801-020	solution
	500 rxn 20 $\mu\ell$	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn 20 $\mu\ell$	801-021	solution
	500 rxn 20 $\mu\ell$	801-051	

Products	Size	Cat. No.	Type

GeneAll® Protein series

ProtinEx™ Animal cell / tissue	100 ml	701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml × 10 tubes	751-001	solution

GeneAll® STEADi™ *for automatic nucleic acid purification*

12 Instrument		GST012	system
24 Instrument		GST024	system
Genomic DNA Cell / Tissue	96	401-104	kit
Genomic DNA Blood	96	402-105	kit
Total RNA	96	404-304	kit
Viral DNA / RNA	96	405-322	kit
CFC Seed DNA / RNA	96	406-C02	kit
Genomic DNA Plant	96	407-117	kit
Soil DNA	96	408-114	kit

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GeneAll

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