Ver 1.0

Cat.No. 306-150

Allspin™

For total DNA & RNA isolation from tissues and cultured cells

TOTAL DNA/RNA PURIFICATION HANDBOOK

GeneAll

Customer & Technical Support

Do not hesitate to ask us any question.

We thank you for any comment or advice.

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This protocol handbook is included in :

GeneAll® Allspin™ (306-150) total DNA/RNA purification Kit

Visit www.geneall.com or www.geneall.co.kr for FAQ, QnA and more information.

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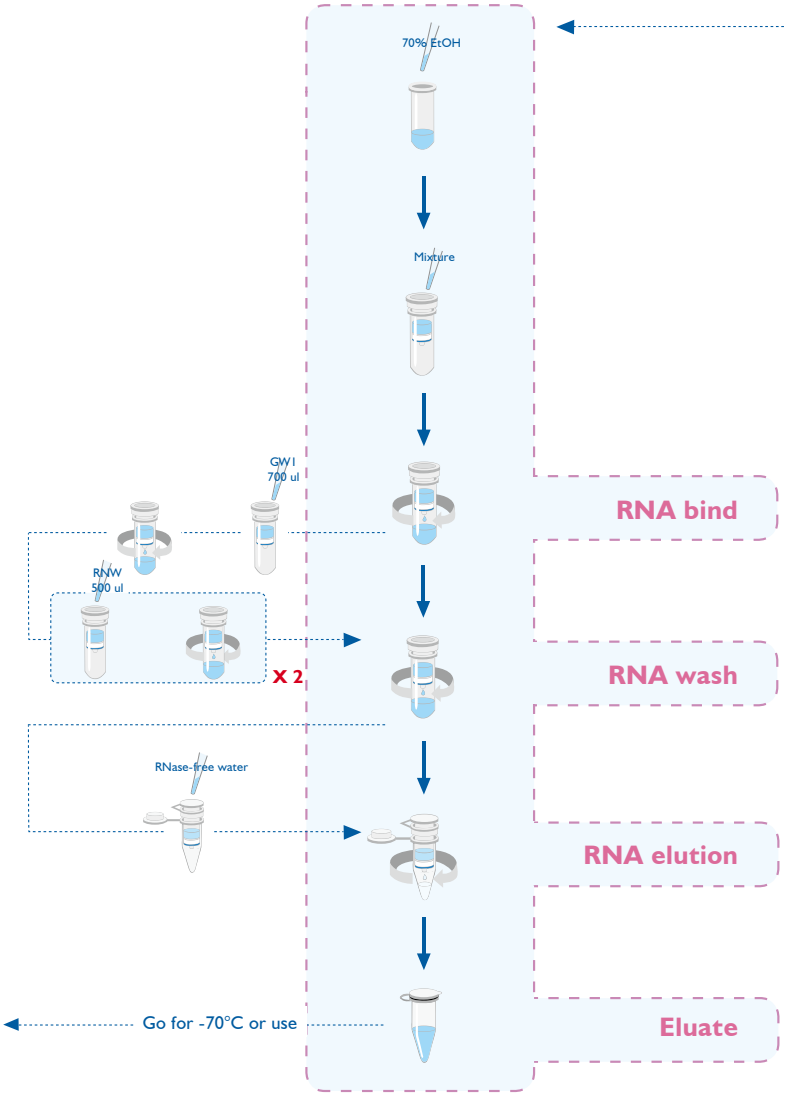
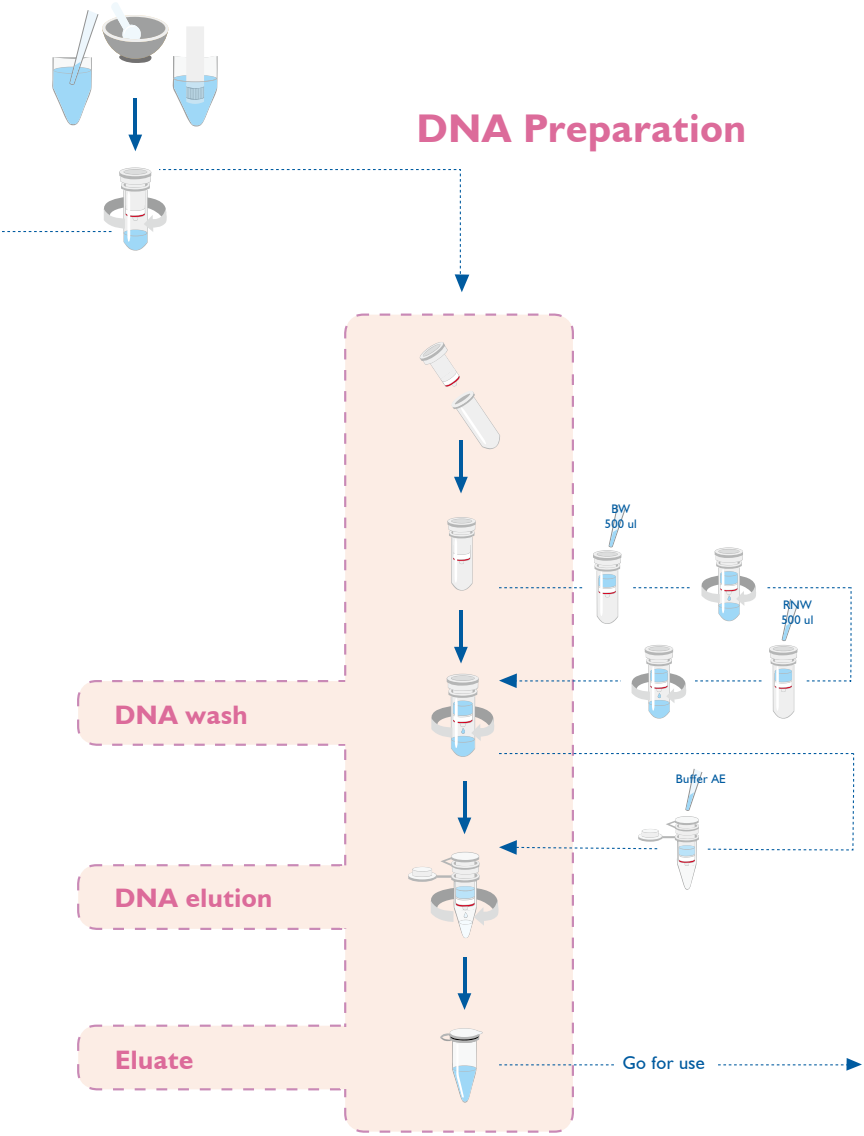
Brief protocol

RNA Preparation



DNA Preparation



Allspin™ total DNA/RNA Purification Kit
Kit Contents

50 preps
Cat.No. 306-150

Components	Quantity	Storage
Buffer CTL	45 ml	Room temperature
Buffer GW1	45 ml	
Buffer BW	30 ml	
Buffer RNW	50 ml x 2	
Buffer AE	15 ml	
RNase-free water	15 ml	
GeneAll® Column type B (red ring) *(with collection tube)*	50	
GeneAll® Column type W (blue ring) *(with collection tube)*	50	
2 ml collection tube without column	50	
1.5 ml microcentrifuge tube	100	

Materials Not Provided

Reagents

- Tissue storage buffer to protect RNA from RNase
- 100 % ethanol, ACS grade or better

Disposable materials

- RNase-free pipet tips
- Disposable gloves

Equipments

- Equipment for homogenizing solid tissue
- Microcentrifuge
- Suitable protector (ex; lab coat, disposable gloves, goggles, etc)

Quality Control

Allspin™ total DNA/RNA Purificaion Kit is manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. For consistency of product, the quality certification process is carried out from lot to lot thoroughly and only the qualified is approved to be delivered.

Storage Conditions

Allspin™ total DNA/RNA Purificaion Kit should be stored at room temperature. All components are stable for 1 year.

User Precautions

The buffers included in Allspin™ total DNA/RNA Purificaion Kit contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions. In case of contact, wash immediately with plenty of water and seek medical advice.
Buffer CTL, GW1, and BW contain chaotropes. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Product Disclaimer

Allspin™ total DNA/RNA Purificaion Kit is for research use only, not for use in diagnostic procedure.

Product Specifications

Specification	Column type B for DNA	Column type W for RNA
Type	Spin	Spin
Maximum amount of starting samples	~ 30 mg tissues or ~ 1 x 10^7 cells	~ 30 mg tissues or ~ 1 x 10^7 cells
Maximum loading volume	~ 700 ul	~ 700 ul
Minimum elution volume	~ 50 ul	~ 30 ul
Maximum binding capacity	~ 100 ug	~ 100 ug
Nucleic acid binding size	~50 kbp	>200 nucleotides

Preventing RNase Contamination

RNase can be introduced accidentally into a RNA preparation. Wear disposable gloves always, because skin often contains bacteria that can be a source of RNase. Use sterile, disposable plasticwares and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase on shared equipment.

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

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

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 by individual but successive procedure using column B and column W respectively. Alternatively, both DNA and RNA can be co-purified into a single tube by the modified procedure at appendix A. 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 nucleoties.

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 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 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 RNase-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

1. Harvest cell samples in a 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 minutes, 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 minutes, then discard the culture medium.

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

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

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

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

This step allows nucleoprotein complexes to completely dissociate.

4. **Transfer the lysate to a mini spin column (type B, red ring).**
Maximun volume is 700 ul and DNA is bound to membrane through this step.

5. **Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature. Transfer the column to a new 2 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 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 ul) 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 ul) including any precipitate to a mini spin column (type W, blue ring).**

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

9. Add 700 ul of buffer GW1 to the mini spin column.

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

11. Add 500 ul of buffer RNW to the mini spin column.

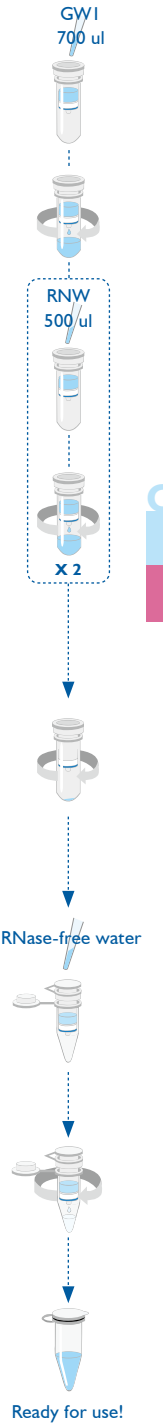
12. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.
Discard the pass-through and reinsert the mini spin column back into the same tube.

13. Repeat the step 11 and 12.

14. Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin 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 ul of RNase-free water to the center of the membrane in the mini spin column.
According to the expected yield, the volume of eluent can be adjusted.

16. Centrifuge at ≥ 10,000 x g for 1 minute 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 ul of buffer BW to the mini spin column.

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

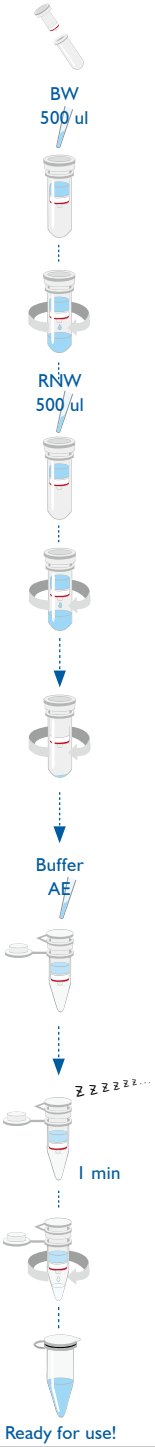
19. Add 500 ul of buffer RNW to the mini spin column.

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

21. Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin 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 ul of buffer AE to the center of the membrane in the mini spin column. Let it stand for 1 minute.
According to the expected yield, the volume of eluent can be adjusted.

23. Centrifuge at ≥ 10,000 x g for 1 minute 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 (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water is higher than 7.0.

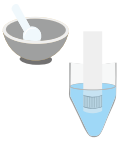


Allspin™ total DNA/RNA Purificaion Kit

PROTOCOL
for animal tissues

1. Harvest tissue samples in a tube.

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



2. Add 350 ul of buffer CTL to the tube and distrupt and homogenize the sample by homogenization.

Before starting, add ß-mercaptoethanol (10 ul per 1 ml) to buffer CTL.

Homogenize ~ 20 mg of tissue samples in 350 ul 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 column in subsequent steps.

3. Incubate the lysate for 10 minutes at room temperature.

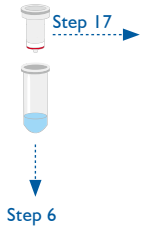
This step allows nucleoprotein complexes to completely dissociate and reduces the generated foam.

4. Centrifuge at maximum speed for 3 minutes at room temperature and carefully transfer the supernatant to a mini spin column (type B, red ring).

This step can help avoid clogging of the mini spin column caused by not clarified insoluble particles.
Maximun volume is 700 ul and DNA is bound to membrane through this step.

5. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.
Transfer the column to a new 2 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 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 ul) 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 ul) including any precipitate to a mini spin column (type W, blue ring).

8. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.

Discard the pass-through and reinsert the mini spin column back into the same tube.

9. Add 700 ul of buffer GW1 to the mini spin column.

10. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.

Discard the pass-through and reinsert the mini spin column back into the same tube.

11. Add 500 ul of buffer RNW to the mini spin column.

12. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.

Discard the pass-through and reinsert the mini spin column back into the same tube.

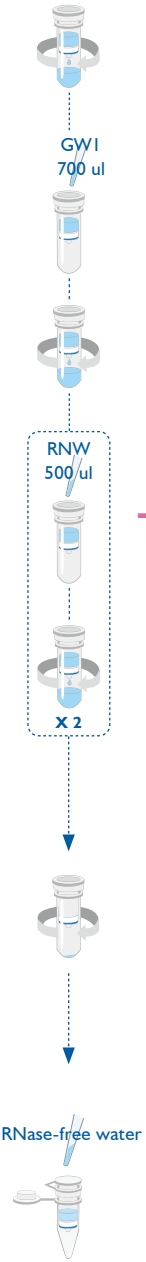
13. Repeat the step 11 and 12.

14. Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin 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 ul of RNase-free water to the center of the membrane in the mini spin column.

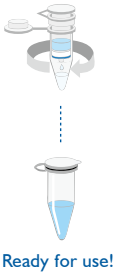
According to the expected yield, the volume of eluent can be adjusted.



16. Centrifuge at ≥ 10,000 x g for 1 minute 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 following the manufacture's instruction manual.



Genomic DNA purification (Red ring column step)

17. Add 500 ul of buffer BW to the mini spin column.

18. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.

Discard the pass-through and reinsert the mini spin column back into the same tube.

19. Add 500 ul of buffer RNW to the mini spin column.

20. Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.

Discard the pass-through and reinsert the mini spin column back into the same tube.

21. Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin column to a new 1.5 ml microcentrifuge tube (provided).

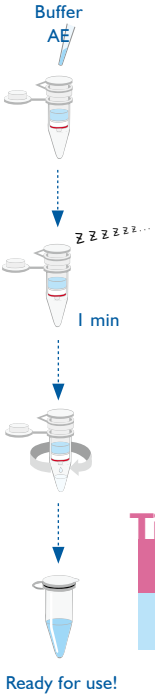
Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

22. Add 100 ul of buffer AE to the center of the membrane in the mini spin column. Let it stand for 1 minute.

According to the expected yield, the volume of eluent can be adjusted.

23. Centrifuge at ≥ 10,000 x g for 1 minute 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 (>pH7.0) or Tris-Cl (>pH8.5). When using water for elution, make sure the pH of water is higher than 7.0.



Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield	**Sample not homogenized completely**	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.
	Culture media not completely removed	Remaining culture media affect lysis and binding condition of buffer CTL.
Column clogging	**Sample not homogenized completely**	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 $A_{260/280}$	**Sample not homogenized completely**	Be sure to incubate after homogenization. Confirm the completely lysed sample in buffer CTL.
High $A_{260/280}$ 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.
High $A_{260/230}$ in DNA eluate	**Water used to dilute DNA or to make blank for $A_{260/230}$ measurement**	Use buffer AE to dilute sample and to make blank before measure purity, If buffer AE has been used for elution.
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 Purificaion Kit manual.

Facts	Possible Causes	Suggestions
DNA contamination in RNA eluate	**Sample has high DNA mass**	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 starting sample**	Reduce the amount of starting sample. Especially for tissue sample, use the correct amount of starting sample.
	Ethanol is added to the lysate	Ethanol should be added to bind RNA. Be sure to apply the only lysate in buffer CTL to DNA bind.
RNA degradation	**Sample manipulated too much 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.
	Reagent or disposable is not RNase-free	Make sure to use RNase free products only.
DNA degradation	**Starting sample is too old or mis-stored**	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.

Facts	Possible Causes	Suggestions
Eluate does not per- from well in downstream application	**Residual ethanol remains in eluate**	To remove any residual ethanol included in buffer RNW from mini spin column membrane, centrifuge again for complete removal of ethanol.
	Buffer BW and RNW used 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 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 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 minutes, 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 minutes, then discard the culture medium.
   * Do not wash cells before lysing with buffer CTL as this may contribute to mRNA degradation.

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

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

4. **Add 1 volume (usually 350 ul) of 100% ethanol to the lysate, and mix well by pipetting or vortexing. Do not centrifuge.**

5. **Transfer the mixture (approximately 700 ul) including any precipitate to a mini spin column (type B, red ring).**

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

7. **Add 500 ul of buffer BW to the mini spin column.**

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

9. **Add 500 ul of buffer RNW to the mini spin column.**

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

11. **Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin 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 ul of RNase-free water to the center of the membrane in the mini spin column. Let it stand for 1 minute.**
    According to the expected yield, the volume of eluent can be adjusted.

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

Protocol for tissue samples.

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

2. **Add 350 ul of buffer CTL to the tube and distrupt and homogenize the sample by homogenization.**
   Before starting, add ß-mercaptoethanol (10 ul per 1 ml) to buffer CTL.
   Homogenize ~ 20 mg of tissue samples in 350 ul 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 column in subsequent steps.

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

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

5. **Add 1 volume (usually 350 ul) of 100% ethanol to the lysate, and mix well by pipetting or vortexing. Do not centrifuge.**

6. **Transfer the mixture (approximately 700 ul) including any precipitate to a mini spin column (type B, red ring).**

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

8. **Add 500 ul of buffer BW to the mini spin column.**

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

10. **Add 500 ul of buffer RNW to the mini spin column.**

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

12. **Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin 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 ul of RNase-free water to the center of the membrane in the mini spin column. Let it stand for 1 minute.**
    According to the expected yield, the volume of eluent can be adjusted.

14. **Centrifuge at ≥ 10,000 x g for 1 minute 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 using the absorbance of spectrophotometer at 260 nm. For the convenient measurement, we recommend using the NanoDrop which can also 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 about 40 ug 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 ug / 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 1g agarose in 72 ml water and heat to dissolve thoroughly.
2. Cool to 60°C.
3. Add 10 ml of 10 X MOPS buffer, 18 ml of 37% formaldehyde, and 1 ul 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 1 X MOPS running buffer to cover the gel.

Prepare the RNA sample

1. Make the mixture.
   ? ul RNA (up to 20 ug)
   2 ul 10 X MOPS electrophoresis buffer
   4 ul formaldehyde
   10 ul formamide
2. Incubate the mixture for 15 minutes at 65°C.
3. Chill the sample for 5 minutes in ice.
4. Add 2 ul of 10 X formaldehyde gel-loading dye to the mixture.
5. Load the mixture in a denaturing gel which is covered with a sufficient 1 X 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

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

- 10 X 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

Products	Size	Type	Cat. No.
GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*			
Plasmid Rapidprep	50 200	mini / spin	100-150 100-102
GeneAll® *Exprep™* *for preparation of plasmid DNA*			
Plasmid SV mini	50 200 1,000	spin / vacuum	101-150 101-102 101-111
Plasmid SV Midi**	26 50 100	spin / vacuum	101-226 101-250 101-201
Plasmid SV Quick	50 200 1,000	mini / spin	101-050 101-002 101-011
GeneAll® *Exfection™* *for preparation of highly pure plasmid DNA*			
Plasmid LE mini *(Low Endotoxin)*	50 200	spin / vacuum	111-150 111-102
Plasmid LE Midi* *(Low Endotoxin)*	26 100	spin / vacuum	111-226 111-201
Plasmid EF Midi* *(Endotoxin Free)*	20 100	spin	121-220 121-201
GeneAll® *Expin™* *for purification of fragment DNA*			
Gel SV	50 200	mini / spin / vacuum	102-150 102-102
PCR SV	50 200	mini / spin / vacuum	103-150 103-102
CleanUp SV	50 200	mini / spin / vacuum	113-150 113-102
Combo GP	50 200	mini / spin / vacuum	112-150 112-102
GeneAll® *Exgene™* *for isolation of total DNA*			
Tissue SV mini (*plus!*)*	100 250	spin / vacuum	104(9)-101 104(9)-152
Tissue SV Midi (*plus!*)**	26 100	spin / vacuum	104(9)-226 104(9)-201
Tissue SV MAXI (*plus!*)**	10 26	spin / vacuum	104(9)-310 104(9)-326
Blood SV mini	100 250	spin / vacuum	105-101 105-152
Blood SV Midi**	26 100	spin / vacuum	105-226 105-201

Ordering Information

Products	Size	Type	Cat. No.
GeneAll® *Exgene™* *for isolation of total DNA*			
Blood SV MAXI**	10 26	spin / vacuum	105-310 105-326
Cell SV mini	100 250	spin / vacuum	106-101 106-152
Cell SV MAXI**	10 26	spin / vacuum	106-310 106-326
Clinic SV mini	100 250	spin / vacuum	108-101 108-152
Clinic SV Midi	26 100	spin / vacuum	108-226 108-201
Clinic SV MAXI**	10 26	spin / vacuum	108-310 108-326
Plant SV mini	100 250	spin / vacuum	117-101 117-152
Plant SV Midi**	26 100	spin / vacuum	117-226 117-201
Plant SV MAXI**	10 26	spin / vacuum	117-310 117-326
GMO SV mini	50 200	spin / vacuum	107-150 107-102
GeneAll® *GenEx™* *for isolation of total DNA*			
GenEx™ B	100† 500† 100††	mini / solution mini / solution MAXI / solution	220-101 220-105 220-301
GenEx™ C	100† 500† 100††	mini / solution mini / solution MAXI / solution	221-101 221-105 221-301
GenEx™ T	100† 500† 100††	mini / solution mini / solution MAXI / solution	222-101 222-105 222-301

** GeneAll® Tissue SV mini, Midi, and MAXI plus! kit provide the additional methods for the purification from animal whole blood.*
*** GeneAll® SV Midi / MAXI kits require the centrifuge which has a swinging-bucket rotor and ability of 4,000 ~ 5,000 xg.*
† On the basis of DNA purification from 300 ul whole blood, 2 x 10^6 cells or 10 mg animal tissue.
†† On the basis of DNA purification from 10 ml whole blood. 1 x 10^8 cells or 100 mg animal tissue.

Ordering Information

Products	Size	Type	Cat. No.
GeneAll® *RNA Series* *for preparation of total RNA*			
RiboEx™	100 200	solution	301-001 301-002
Hybrid-R™	100	spin	305-101
RiboEx™ LS	100 200	solution	302-001 302-002
Riboclear™	50	spin	303-150
Ribospin™	50	spin	304-150
Ribospin vRD™	50	spin	302-150
Allspin™	50	spin	306-150
GeneAll® *AmpONE™* *for PCR amplification*			
Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	501-025 501-050 501-100
α-Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	502-025 502-050 502-100
Pfu DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	503-025 503-050 503-100
Hot start Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	531-025 531-050 531-100
Clean Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	551-025 551-050 551-100
Clean α-Taq DNA polymerase	250 U 500 U 1,000 U	(2.5 U/μℓ)	552-025 552-050 552-100
Taq Master mix	2x	0.5 ml x 2 tubes	511-010
α-Taq Master mix	2x	0.5 ml x 2 tubes	512-010
Taq Premix	20 μℓ 50 μℓ	96 tubes	521-200 521-500
α-Taq Premix	20 μℓ 50 μℓ	96 tubes	522-200 522-500
dNTP mix	500 μℓ	2.5 mM each	509-020
dNTP set (set of dATP, dCTP, dGTP and dTTP)	1 ml x 4 tubes	100 mM	509-040

* Each dNTP is available

Note

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