Axen™ Total RNA Kit

Product description

Axen™ Total RNA kit provides the simple and fast method for purification of total RNA from various biological samples, such as cultured cells and animal tissues. Highly pure RNA can be isolated in less than 30 minutes without any use of hazardous organic solvents and alcohol precipitation. Purified RNA can be directly used in various downstream applications without any further manipulations.

Kit contents

Contents*			Storage conditions
Cat. No.	**MG-P-012-50**	**MG-P-012-200**	
No. of preparation	50	200	
Mini spin column w/tube	50 ea	200 ea	Room Temperature
Buffer DRB	5 mL	20 mL	
Buffer RAL**	40 mL	150 mL	
Buffer RW	40 mL	155 mL	
Buffer RWA (conc.)***	15 mL	28 mL x 2	
Nuclease Free Water	15 mL	30 mL	
DNase I solution (2 U/uL)	110 uL	420 uL	- 20℃

* All components of this kit should be stored at room temperature (15~25°C). Long exposure to heat source can deteriorate the performance of kit significantly.

** During shipment or storage under cold ambient condition, a precipitate can be formed in buffer RAL. Heat the bottle at 20~40°C to dissolve completely before use.

*** Buffer RWA is provided as concentrate. Ethanol must be added before first use as the indication on the bottle labels.

Product use limitations

Axen™ Total RNA kit is intended for research uses only. This kit is not intended for diagnosis or treatment for human. All due care and attention should be exercised in the handling of the products.

Safety information

Axen™ Total RNA kit contains irritants which are harmful when in contact with skin or eyes, or inhaled or swallowed. Care should be taken when handling this product. Always wear gloves and eye protection, and follow standard safety precautions.

Buffer RAL and buffer RW contain chaotropes which can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Preventing RNase Contamination

RNase can be introduced accidentally during RNA preparation. Various factors such as reagents, air, dust, and human hands or skin, can be the sources of RNase contamination. Always wear disposable gloves and use sterile, disposable plastic wares. Use the pipette reserved for RNA work. Maintain a separate area for RNA work. Carefully clean the surfaces.

Required Materials Not Provided

Reagents	- β-mercaptoethanol, ACS grade or better - 70% ethanol, ACS grade or better - Absolute ethanol, ACS grade or better
Disposable materials	- RNase-free pipette tips - Sterile 1.5 mL microcentrifuge tubes
Equipments	- Equipment for homogenizing sample - Devices for liquid handling - Microcentrifuge - Suitable protector (ex; lab coat, disposable gloves, goggles, etc)

Preparation of ethanol-added buffer

Buffer RWA is provided as concentrate. Absolute ethanol (ACS grade or better) should be added before first use as below.

Buffer name	Cat. No.	Volume of contents	Ethanol to be added	The final volume
RWA	MG-P-012-50	15 mL	**60 mL**	75 mL
	MG-P-012-200	28 mL x 2	**112 mL x 2**	140 mL x 2

DNA-free RNA

Most of DNA in the starting sample is removed during the preparation procedure. But, if DNA should be further eliminated from the preparation, it can be removed by treating the eluate with DNase I at page 18 ‘Appendix II’. DNase I solution is provided in this kit. Also, any DNase I solution with a concentration of 1 U/uL or more can be applied.

Protocol for the preparation of total RNA with on-column DNase I digestion

A. Procedure for animal cultured cells

To check before start,

- Prepare absolute ethanol
- Prepare 'DNase I reaction mixture' as below; *Make this mixture as just before step 6 as possible.*

① Mix DNase I solution with buffer DRB for a preparation as below table.

DNase I stock conc.	DNase I sol. to be added per prep.	Buffer DRB to be added per prep.	Final volume of mixture
1 U/uL	4 uL	66 uL	70 uL
2 U/uL*	**2 uL**	**68 uL**	**70 uL**
5 U/uL	1 uL	69 uL	70 uL

* Concentration of provided DNase I

② Mix gently by pipetting without vortex.

③ Keep the mixture on ice until use.

1. Harvest cells in a tube as below.

➢ *Cells grown in monolayer*

Harvest 5 x 10^6 cells carefully using scraper, pellet cells by centrifugation at low speed (below 800 xg) for 5 minutes, and then discard the culture medium.

➢ *Cells grown in suspension*

Pellet 5 x 10^6 cells by centrifugation at low speed (below 800 x g) for 5 minutes, and discard the culture medium.

- *Do not wash the cells before lysing with buffer RAL as this may cause mRNA degradation.*

2. **Add 350 uL of buffer RAL to the tube and lyse the sample by pipetting or micro-homogenizer**

   - *If the number of cells exceeds 5 x 10^6, add 700 uL of buffer RAL. Do not apply more than 1 x 10^7 cells for 1 preparation. An insufficient lysis due to large sample may result in poor result.*

3. **Add 1 volume of 70% ethanol to the lysate and mix well by pipetting.**

   - *Do not centrifuge at this step.*

4. **Transfer 700 uL of the mixture into a mini spin column and centrifuge at 10,000 xg for 1 min at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**

   - *If the mixture remains, repeat step 4 with the remaining mixture.*

5. **Apply 350 uL of buffer RW to the spin column, centrifuge at 10,000 xg for 30 secs at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**

6. **Apply 70 uL of DNase I reaction mixture on to the center of the spin column membrane and incubate for 10 mins at room temperature.**

   - *Refer to the section of 'To check before start' for the preparation of DNase I reaction mixture*

7. **Apply 350 uL of buffer RW to the spin column, centrifuge at 10,000 xg for 30 secs at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**

8. **Apply 600 uL of buffer RWA to the spin column, centrifuge at full speed for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

9. **Apply 600 uL of absolute ethanol to the spin column, centrifuge at full speed for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

10. **Centrifuge at full speed for 1 min for removing the residual wash buffer and transfer the spin column into a new 1.5 mL microcentrifuge tube.**

    - *Residual wash buffer may interfere with downstream applications. Care must be taken not to be contaminated by the carryover of buffer RWA.*

11. **Apply 50 uL of Nuclease Free Water to the center of spin column membrane and let it stand for 1 min.**

    - *Elution volume can be adjusted according to the purpose of experiment.*
    - *Using lesser amount than 50 uL will decrease the total RNA yield but increase the concentration of RNA. Note that at least 30 uL of eluent must be applied because the lesser eluent will not soak the membrane entirely, followed by poor or unpredictable result.*

12. **Centrifuge at full speed for 1 min for eluting the RNA.**

    - *Purified RNA can be temporarily stored at 4°C for an immediate analysis, but it is strongly recommended to store at -70°C for long-term storage.*

B. Procedure for animal tissues

To check before start,

- This protocol is suitable for fresh, frozen, or reagent-stabilized tissue sample.
- Prepare 1% β-mercaptoethanol(BME) in buffer RAL. Otherwise, you can add 1% BME into buffer RAL on every single preparation.
- Prepare absolute ethanol
- Prepare 'DNase I reaction mixture' as below; *Make this mixture as just before step 6 as possible*.

① Mix DNase I solution with buffer DRB for a preparation as below table.

DNase I stock conc.	DNase I sol. to be added per prep.	Buffer DRB to be added per prep.	Final volume of mixture
1 U/uL	4 uL	66 uL	70 uL
2 U/uL*	**2 uL**	**68 uL**	**70 uL**
5 U/uL	1 uL	69 uL	70 uL

***** Concentration of provided DNase I

② Mix gently by pipetting without vortex.

③ Keep the mixture on ice until use.

1. Homogenize up to 20 mg of animal tissue as one of the methods described below.

- *It is essential to homogenize thoroughly the tissue in buffer RAL and to lyse completely the sample.*
- *When using the fiber-rich tissue samples like heart and muscle, it is strongly recommended to apply lesser sample than 10 mg. The larger sample may not be lysed completely, and this will bring up poor result.*
- *Use a double volume (700 uL) of buffer RAL for the larger sample than 20 mg, but it should not exceed 30 mg per preparation.*

A. **Grind the tissue sample to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle. Put up to 20 mg of the powdered tissue into 1.5 mL microcentrifuge tube. Add 350 ul of buffer RAL (including 1% BME) and pulse-vortex for 30 secs.**

B. **Homogenize up to 20 mg of the tissue sample in 350 ul of buffer RAL (including 1% BME) using a hand-held or a rotor-stator homogenizer.**

C. **Pulverize up to 20 mg of the tissue sample in 2.0 mL tube using bead-beater. Add 350 ul of buffer RAL (including 1% BME) and pulse-vortex for 30 secs.**
Follow the instruction manuals for usage of the bead-beating instruments.

2. **Centrifuge at 10,000 xg for 2 mins and transfer carefully the supernatant to a new 1.5 mL microcentrifuge tube(not provided).**

   - *This step helps remove the debris of the homogenate and not clog a column membrane by the debris.*

3. **Add 1 volume of 70% ethanol to the lysate and mix well by pipetting.**

   - *Do not centrifuge at this step.*

4. **Transfer 700 uL of the mixture into a mini spin column and centrifuge at 10,000 xg for 1 min at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**

   - *If the mixture remains, repeat step 4 with the remaining mixture.*
   - *Make sure that any residual lysate does not remain in the column after centrifugation. If a residual lysate has remained, centrifuge again at full speed until all of the solution has passed though completely.*

5. **Apply 350 uL of buffer RW to the spin column, centrifuge at 10,000 xg for 30 secs at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**

6. **Apply 70 uL of DNase I reaction mixture on to the center of the spin column membrane and incubate for 10 mins at room temperature.**

   - *Refer to the section of 'To check before start' for the preparation of DNase I reaction mixture*

7. **Apply 350 uL of buffer RW to the spin column, centrifuge at 10,000 xg for 30 secs at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**

8. **Apply 600 uL of buffer RWA to the spin column, centrifuge at full speed for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

9. **Apply 600 uL of absolute ethanol to the spin column, centrifuge at full speed for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

10. **Centrifuge at full speed for 1 min for removing the residual wash buffer and transfer the spin column into a new 1.5 mL microcentrifuge tube.**

    - *Residual wash buffer may interfere with downstream applications. Care must be taken not to be contaminated by the carryover of buffer RWA.*

11. **Apply 50 uL of Nuclease Free Water to the center of spin column membrane and let it stand for 1 min.**

    - *Elution volume can be adjusted according to the purpose of experiment.*
    - *Using lesser amount than 50 uL will decrease the total RNA yield but increase the concentration of RNA. Note that at least 30 uL of eluent must be applied because the lesser eluent will not soak the membrane entirely, followed by poor or unpredictable result.*

12. **Centrifuge at full speed for 1 min for eluting the RNA.**

    - *Purified RNA can be temporarily stored at 4°C for an immediate analysis, but it is strongly recommended to store at -70°C for long-term storage.*

Trouble shooting guide

Facts	*Possible causes*	Recommendations
Low recovery of RNA	*Sample not homogenized completely*	Insufficient disruption can lead to decrease in yield of total RNA and this may be attributed to several reasons; - Insufficient mixing with buffer RAL - Too much mass in the starting sample - Poor pulverization of sample Ensure the complete homogenization of the sample with buffer RAL.
	Too much starting materials	Too much starting materials will bring about inefficient lysis, followed by poor RNA yields. Reduce the amount of starting material as described on procedure.
	Poor quality of starting material	Use a freshly harvested sample if possible. The harvested sample should be handled under low temperature before addition of buffer RAL.
	Ethanol was not added to the wash buffer	Buffer RWA is provided as concentrate. Ethanol must be added to this buffer before first use. If not, the result will be significantly poor.
	Culture media not completely removed	Culture media affect the lysis and the binding efficiency. Discard the culture media as completely as possible when harvest.
Column clogged	*Sample not homogenized completely*	Refer to the same item at ‘Low recovery of RNA’ section.
	Too much starting materials	Refer to the same item at ‘Low recovery of RNA’ section.
RNA degraded	*Inappropriate handling of starting materials*	Starting sample should be quickly treated under low temperature. Higher temperature or retarded processing would be a cause of degradation.
	Poor quality of starting material	Refer to the same item at ‘Low recovery of RNA’ section.

	RNase contamination	RNase can be introduced accidentally into a preparation at any steps. Always wear disposable gloves and use RNase-free plasticwares. Do not use shared equipment if possible.
DNA contamination	*Incorrect treatment of DNase I reaction mixture*	DNase I reaction mixture should be pipetted onto the center of the spin column membrane for proper enzymatic reactions.
Enzymatic reaction is not performed well with purified RNA	*Salt carryover in eluate*	Ensure that washing steps are carried out just in accordance with the protocols. Additional washing steps with absolute ethanol may help remove salts from the membrane.
	Residual ethanol in eluate	The spin column membrane should be dried completely before eluting. Perform additional centrifugation to dry the membrane, if needed. Do NOT incubate the column at high temperature.

Appendix I.

Protocol for preparation of total RNA without DNase I treatment

A. Procedure for animal cultured cells

To check before start,

- Prepare absolute ethanol

1. **Harvest cells in a tube as below.**
   - *Cells grown in monolayer*
     Harvest 5 x 10^6 cells carefully using scraper, pellet cells by centrifugation at low speed (below 800 x g) for 5 minutes, and then discard the culture medium.
   - *Cells grown in suspension*
     Pellet 5 x 10^6 cells by centrifugation at low speed (below 800 x g) for 5 minutes, and discard the culture medium.
   - *Do not wash the cells before lysing with buffer RAL as this may cause mRNA degradation.*
2. **Add 350 uL of buffer RAL to the tube and lyse the sample by pipetting or micro-homogenizer**
   - *If the number of cells exceeds 5 x 10^6, add 700 uL of buffer RAL. Do not apply more than 1 x 10^7 cells for 1 preparation. An insufficient lysis due to large sample may result in poor result.*
3. **Add 1 volume of 70% ethanol to the lysate and mix well by pipetting.**
   - *Do not centrifuge at this step.*
4. **Transfer 700 uL of the mixture into a mini spin column and centrifuge at 10,000 xg for 1 min at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**
   - *If the mixture remains, repeat step 4 with the remaining mixture.*

5. **Apply 600 uL of buffer RW to the spin column, centrifuge at 10,000 xg for 30 secs at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**
6. **Apply 600 uL of buffer RWA to the spin column, centrifuge at full speed for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
7. **Apply 600 uL of absolute ethanol to the spin column, centrifuge at full speed for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
8. **Centrifuge at full speed for 1 min for removing the residual wash buffer and transfer the spin column into a new 1.5 mL microcentrifuge tube.**
    - *Residual wash buffer may interfere with downstream applications. Care must be taken not to be contaminated by the carryover of buffer RWA.*
9. **Apply 50 uL of Nuclease Free Water to the center of spin column membrane and let it stand for 1 min.**
    - *Elution volume can be adjusted according to the purpose of experiment.*
    - *Using lesser amount than 50 uL will decrease the total RNA yield but increase the concentration of RNA. Note that at least 30 uL of eluent must be applied because the lesser eluent will not soak the membrane entirely, followed by poor or unpredictable result.*
10. **Centrifuge at full speed for 1 min for eluting the RNA.**
    - *Purified RNA can be temporarily stored at 4°C for an immediate analysis, but it is strongly recommended to store at -70°C for long-term storage.*

B. Procedure for animal tissues

To check before start,

- This protocol is suitable for fresh, frozen, or reagent-stabilized tissue sample.
- Prepare 1% β-mercaptoethanol(BME) in buffer RAL. Otherwise, you can add 1% BME into buffer RAL on every single preparation.
- Prepare absolute ethanol

1. Homogenize up to 20 mg of animal tissue as one of the methods described below.

- *It is essential to homogenize thoroughly the tissue in buffer RAL and to lyse completely the sample.*
- *When using the fiber-rich tissue samples like heart and muscle, it is strongly recommended to apply lesser sample than 10 mg. The larger sample may not be lysed completely, and this will bring up poor result.*
- *Use a double volume (700 uL) of buffer RAL for the larger sample than 20 mg, but it should not exceed 30 mg per preparation.*

A. Grind the tissue sample to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle. Put up to 20 mg of the powdered tissue into 1.5 mL microcentrifuge tube. Add 350 ul of buffer RAL (including 1% BME) and pulse-vortex for 30 secs.

B. Homogenize up to 20 mg of the tissue sample in 350 ul of buffer RAL (including 1% BME) using a hand-held or a rotor-stator homogenizer.

C. Pulverize up to 20 mg of the tissue sample in 2.0 mL tube using bead-beater. Add 350 ul of buffer RAL (including 1% BME) and pulse-vortex for 30 secs.
Follow the instruction manuals for usage of the bead-beating instruments.

2. **Centrifuge at 10,000 xg for 2 mins and transfer carefully the supernatant to a new 1.5 mL microcentrifuge tube(not provided).**

   - *This step helps remove the debris of the homogenate and not clog a column membrane by the debris.*

3. **Add 1 volume of 70% ethanol to the lysate and mix well by pipetting.**

   - *Do not centrifuge at this step.*

4. **Transfer 700 uL of the mixture into a mini spin column and centrifuge at 10,000 xg for 1 min at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**

   - *If the mixture remains, repeat step 4 with the remaining mixture.*
   - *Make sure that any residual lysate does not remain in the column after centrifugation. If a residual lysate has remained, centrifuge again at full speed until all of the solution has passed though completely.*

5. **Apply 600 uL of buffer RW to the spin column, centrifuge at 10,000 xg for 30 secs at room temperature, empty the collecting tube and re-insert the spin column back to the tube.**

6. **Apply 600 uL of buffer RWA to the spin column, centrifuge at full speed for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

7. **Apply 600 uL of absolute ethanol to the spin column, centrifuge at full speed for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

8. **Centrifuge at full speed for 1 min for removing the residual wash buffer and transfer the spin column into a new 1.5 mL microcentrifuge tube.**

   - *Residual wash buffer may interfere with downstream applications. Care must be taken not to be contaminated by the carryover of buffer RWA.*

9. Apply 50 uL of Nuclease Free Water to the center of spin column membrane and let it stand for 1 min.

- *Elution volume can be adjusted according to the purpose of experiment.*
- *Using lesser amount than 50 uL will decrease the total RNA yield but increase the concentration of RNA. Note that at least 30 uL of eluent must be applied because the lesser eluent will not soak the membrane entirely, followed by poor or unpredictable result.*

10. Centrifuge at full speed for 1 min for eluting the RNA.

- *Purified RNA can be temporarily stored at 4°C for an immediate analysis, but it is strongly recommended to store at -70°C for long-term storage.*

Appendix II.
Procedure for DNase I treatment in RNA eluate

To check before start,

- Prepare 0.25M EDTA and 75°C water bath or dry bath.

1. **Prepare the reaction mixture freshly in a 1.5 mL tube as below;**
   - 50 uL RNA eluate
   - 5 uL buffer DRB
   - 2U DNase I solution
2. **Incubate the mixture for 10 mins at room temperature.**
3. **Proceed to next step for direct use or proceed to 'Appendix III' for clean-up the reaction mixture**
   - *It is recommended to proceed to clean-up procedure at '*Appendix III'*, because the inactivation process of DNase I at step 5 can deteriorate the quality of RNA.*
4. **Add 1 uL of 0.25 M EDTA to the tube.**
5. **Inactivate DNase I by incubating at 75°C for 10 mins.**

Appendix III.
Procedure for RNA clean-up

1. **Adjust the sample volume to 100 uL with Nuclease Free Water.**
2. **Add 350 uL of buffer RAL and mix thoroughly.**
3. **Add 250 uL of absolute ethanol to the tube and mix well by pipetting.**
4. **Transfer all of the mixture into a mini spin column and centrifuge at 10,000 xg for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
5. **Apply 600 uL of buffer RWA to the spin column, centrifuge at full speed for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
6. **Repeat the step 5 once.**
7. **Centrifuge at full speed for 1 min for removing the residual wash buffer and transfer the spin column into a new 1.5 mL microcentrifuge tube.**
   - *Residual wash buffer may interfere with downstream applications. Care must be taken not to be contaminated by the carryover of buffer RWA.*
8. **Apply 50 uL of Nuclease Free Water to the center of spin column membrane and let it stand for 1 min.**
   - *Elution volume can be adjusted according to the purpose of experiment.*
   - *Using lesser amount than 50 uL will decrease the total RNA yield but increase the concentration of RNA. Note that at least 30 uL of eluent must be applied because the lesser eluent will not soak the membrane entirely, followed by poor or unpredictable result.*
9. **Centrifuge at full speed for 1 min for eluting the RNA.**
   - *Purified RNA can be temporarily stored at 4°C for an immediate analysis, but it is strongly recommended to store at -70°C for long-term storage.*


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