Axen™ Plant RNA mini Kit

Product description

Axen™ Plant RNA mini kit is designed for purification of total RNA from various plant tissues such as leaves, stems, and roots. This kit provides the optimized buffer and spin column, which is effective for isolating intact plant RNA without contaminating of plant secondary metabolites, such as polyphenols and polysaccharides. All components of this kit are ready for use, and whole procedure takes just 25 minutes for complete. Purified RNA is suitable for cDNA synthesis, RT-PCR, blotting and other downstream applications.

Kit contents

Contents*			**Storage conditions**
Cat. No.	**MG-P-014-50**	**MG-P-014-200**	
No. of preparation	50	200	
Mini spin column w/tube	50 ea	200 ea	Room Temperature
DebX™ column w/tube	50 ea	200 ea	
Buffer DRB	5 mL	20 mL	
Buffer RPL**	22 mL	90 mL	
Buffer RW1 (conc.)**,***	30 mL	110 mL	
Buffer RW2 (conc.)***	18 mL	32 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 RPL or RW1. Heat the bottle at 20~40°C to dissolve completely before use.

*** Buffer RW1 and RW2 are provided as concentrate. Ethanol must be added before first use as the indication on the bottle labels.

**** DNase I solution is delivered by low temperature and it should be stored at -20°C on arrival for conservation of activity.

Product use limitations

Axen™ Plant RNA mini 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™ Plant RNA mini 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 RPL and RW1 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	- 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 working buffer

Buffer RW1 and RW2 are 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
RW1	MG-P-014-50	30 mL	**30 mL**	60 mL
	MG-P-014-200	110 mL	**110 mL**	220 mL
RW2	MG-P-014-50	18 mL	**72 mL**	90 mL
	MG-P-014-200	32 mL x 2	**128 mL x 2**	160 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 9, ‘Appendix’. DNase I solution is provided in this kit. Also, any DNase I solution with a concentration of 1 U/uL or higher can be applied.

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

To check before start

- All centrifugation steps should be performed at full speed (>10,000 xg, 10,000 ~ 14,000 rpm) in a microcentrifuge at room temperature.
- Prepare 'DNase I reaction mixture' as below;

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.

- *Make this mixture as just before step 9 as possible.*

1. Place 30 ~ 50 mg of ground plant tissue into a 1.5 mL tube.

- *Too much sample can cause the column-clogging, followed by poor result. Up to 100 mg can be applied per prep.*
- *Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Quick and complete pulverization at low temperature is essential for good result.*
- *Mortar and pestle is a good conventional method for pulverizing, but other methods like a bead-beating instrument or a rotor-stator homogenizer can be good alternatives. Make sure to treat the sample at low temperature always. Follow the instruction manuals for those methods.*

2. **Add 400 uL of buffer RPL to the tube and vortex vigorously to get homogenate.**
   - *It is essetial to make the lysate homogenized for good result.*
3. **Incubate 3 mins at room temperature.**
4. **Transfer all of the lysate into a Debex™ column and centrifuge for 1 min.**
   - *When transferring the thick lysate, it will be helpful to use a 'Wide-bore tip' or to use the tip cut off the end of pipette tip with sterile scissors.*
5. **Transfer carefully 300 uL of the supernatant in the collecting tube into a new 1.5 mL microcentrifuge tube.**
   - *Be careful not to disturb the bottom pellet when transferring.*
6. **Add 1 transferred volume (300 uL) of 70% ethanol to the tube and mix thoroughly by pipetting or inverting.**
7. **Transfer all of the mixture into a spin column and centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
8. **Apply 500 uL of buffer RW1 to the spin column, centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**
9. **Apply 70 uL of DNase I reaction mixture to the center of the spin column and incubate for 10 mins at room temperature.**
   - *Refer to 'To check before start' above for preparation of DNase I reaction mixture.*
10. **Apply 500 uL of buffer RW1 to the spin column and let it stand for 2 mins.**
    - *The components of buffer RW1 will inactivate DNase I during incubation.*

11. **Centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

12. **Apply 750 uL of buffer RW2 to the spin column, centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

13. **Repeat the step 12 once.**

14. **Centrifuge the tube 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 RW2.*

15. **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.*

16. **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.*

Protocol for preparation of plant RNA without DNase I treatment

To check before start

- All centrifugation steps should be performed at full speed (>10,000 xg, 10,000 ~ 14,000 rpm) in a microcentrifuge at room temperature.

1. **Place 30 ~ 50 mg of ground plant tissue into a 1.5 mL tube.**
   - *Too much sample can cause the column-clogging, followed by poor result. Up to 100 mg can be applied per prep.*
   - *Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen. Quick and complete pulverization at low temperature is essential for good result.*
   - *Mortar and pestle is a good conventional method for pulverizing, but other methods like a bead-beating instrument or a rotor-stator homogenizer can be good alternatives. Make sure to treat the sample at low temperature always. Follow the instruction manuals for those methods.*
2. **Add 400 uL of buffer RPL to the tube and vortex vigorously to get homogenate.**
   - *It is essetial to make the lysate homogenized for good result.*
3. **Incubate 3 mins at room temperature.**
4. **Transfer all of the lysate into a Debex™ column and centrifuge for 1 min.**
   - *When transferring the thick lysate, it will be helpful to use a 'Wide-bore tip' or to use the tip cut off the end of pipette tip with sterile scissors.*
5. **Transfer carefully 300 uL of the supernatant in the collecting tube into a new 1.5 mL microcentrifuge tube.**
   - *Be careful not to disturb the bottom pellet when transferring.*

6. **Add 1 transferred volume (300 uL) of 70% ethanol to the tube and mix thoroughly by pipetting or inverting.**

7. **Transfer all of the mixture into a spin column and centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

8. **Apply 600 uL of buffer RW1 to the spin column, centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

9. **Apply 750 uL of buffer RW2 to the spin column, centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

10. **Repeat the step 9 once.**

11. **Centrifuge the tube 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 RW2.*

12. **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.*

13. **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.

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 in a 1.5 mL tube as below;**
   - 50 uL RNA eluate
   - 5 uL buffer DRB
   - 1 uL DNase I solution(2 U/uL)
2. **Incubate the mixture for 10 mins at room temperature.**
3. **Proceed to next step for direct use, or clean-up the reaction mixture using APure™ RNA CleanUp kit (H3205/H3225).**
   - ➢ *It is recommended to use RNA CleanUp kit, 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.**

Trouble shooting guide

Facts	*Possible causes*	Recommendations
Low recovery of RNA	*Too much starting materials*	Too much starting materials may bring about inefficient lysis and/or spin column clogging, 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 RPL.
	Insufficient disruption	Pulverizing of the sample is critical step for good result. Improperly disrupted sample will result in poor lysis, followed by poor yield. Pulverize quickly and completely the tissue under liquid nitrogen.
	The lysate not homogenized thoroughly	It is critical for good result to make the lysate homogenized after addition of buffer RPL. And this process should be carried out quickly at low temperature.
	Ethanol was not added to the buffer	Buffers RW1 and RW2 are provided as concentrate. Ethanol must be added to these buffers before first use. If not, the result will be significantly poor.
Clogging of Debex™ column	*Too much starting materials*	Too much starting sample can cause clogging of the Debex™ column. Reduce the amount of starting sample.
	Too much saccharides in the sample	The lysate from the sample containing a lot of saccharides may become very thick after incubation. Before applying to Debex™ column, do the follows: Centrifuge the lysate for 1 min at full speed and apply the supernatant into the Debex™ column.
	Insufficient disruption	Insufficient disruption can lead to clogging of the Debex™ column. Pulverize quickly and completely the tissue to make fine powder.

Clogging of spin column	*Lysate too viscous or sticky*	Reduce the amount of starting sample, or increase the amount of buffers proportionally.
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.
	Improper storage of starting materials	Harvested plant tissue should be stored at -70℃ for later use. RNA will be gradually degraded even at -20℃.
	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	*Too much starting materials*	Large mass of DNA in overloaded sample cannot be efficiently removed by Debex™ column and/or on-column DNase I treatment. Reduce the starting material at next preparation.
	Large DNA mass of starting materials	Some plants have larger mass of DNA than others. In this case, it is recommended to reduce the starting amount or perform the optional DNase I treatment.
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 RW2 washing 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.


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