Axen™ Plant SFGR RNA Kit

Product description

Axen™ Plant SFGR RNA kit is designed for isolation of total RNA from plant seeds, fruits, grains, and root vegetables. 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 polysaccharides and polyphenolics. All components of this kit are ready for use, and whole procedure takes just 30 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-013-50**	**MG-P-013-200**	
No. of preparation	50	200	
Spin column CD w/tube	50 ea	200 ea	Room Temperature
DebX™ column w/tube	50 ea	200 ea	
Buffer DRB	5 mL	20 mL	
Buffer SF1	30 mL	120 mL	
Buffer SF2**	20 mL	70 mL	
Buffer RW1 (conc.)**,***	30 mL	110 mL	
Buffer RW2 (conc.)***	10 mL	40 mL	
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 SF2 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.

Product use limitations

Axen™ Plant SFGR 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™ Plant SFGR 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 SF2 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	- 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 RW1 and RW2 are provided as concentrate. Absolute ethanol (ACS grade or better) should be added as below before first use.

Buffer name	Cat. No.	Volume of contents	Ethanol to be added	The final volume
RW1	MG-P-013-50	30 mL	**30 mL**	60 mL
	MG-P-013-200	110 mL	**110 mL**	220 mL
RW2	MG-P-013-50	10 mL	**40 mL**	50 mL
	MG-P-013-200	40 mL	**160 mL**	200 mL

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 7 ‘Appendix’. 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 preparation of RNA with on-column DNase I digestion

To check before start

- 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.*

- All centrifugation steps should be performed at full speed (>13,000 xg or 12,000 rpm) in a microcentrifuge at room temperature.

1. Place up to 100 mg of ground sample into a 1.5 mL microcentrifuge tube.

- *Grind sample 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 500 uL of buffer SF1 and 10 uL of β-mercaptoethanol to the sample tube and vortex vigorously for 15 secs to get homogenate.**
3. **Incubate the lysate for 3 mins at room temperature.**
4. **Centrifuge for 1 min and transfer 300 uL of the supernatant to a new 1.5 mL microcentrifuge tube.**
   - *When the supernatant is thick after centrifugation, 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. **Add 300 uL of buffer SF2 to the tube, vortex vigorously for 15 secs, and transfer all of the mixture to a DebX™ column.**
6. **Centrifuge for 1 min and transfer 500 uL of the passed-through to a new 1.5 mL microcentrifuge tube.**
   - *Be careful not to disturb the bottom pellet when transferring.*
7. **Add 250 uL of absolute ethanol to the tube and mix thoroughly by inverting.**
   - *A precipitate may form after addition of ethanol, but it does not affect the RNA preparation.*
8. **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.**
   - *Transfer all of the mixture including any precipitates into the spin column.*
9. **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.**

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

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

11. **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.**

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. **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.*

14. **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.*

15. **Centrifuge at full speed for 1 min to elute 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 RNA without DNase I treatment

To check before start

- All centrifugation steps should be performed at full speed (>13,000 xg or 12,000 rpm) in a microcentrifuge at room temperature.

1. **Place up to 100 mg of ground sample into a 1.5 mL microcentrifuge tube.**
   - *Grind sample 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 500 uL of buffer SF1 and 10 uL of β-mercaptoethanol to the sample tube and vortex vigorously for 15 secs to get homogenate.**
3. **Incubate the lysate for 3 mins at room temperature.**
4. **Centrifuge for 1 min and transfer 300 uL of the supernatant to a new 1.5 mL microcentrifuge tube.**
   - *When the supernatant is thick after centrifugation, 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. **Add 300 uL of buffer SF2 to the tube, vortex vigorously for 15 secs, and transfer all of the mixture to a DebX™ column.**

6. **Centrifuge for 1 min and transfer 500 uL of the passed-through to a new 1.5 mL microcentrifuge tube.**
   - *Be careful not to disturb the bottom pellet when transferring.*
7. **Add 250 uL of absolute ethanol to the tube and mix thoroughly by inverting.**
   - *A precipitate may form after addition of ethanol, but it does not affect the RNA preparation.*
8. **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.**
   - *Transfer all of the mixture including any precipitates into the spin column.*
9. **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.**
10. **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.**
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 to elute 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 RNA CleanUp kit.**
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 SF1.
	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.
	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 DebX™ column	*Insufficient disruption*	Insufficient disruption can lead to clogging of the DebX™ column. Pulverize quickly and completely the tissue to make it 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.
	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 DebX™ 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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