Axen™ Plant DNA mini Kit

Product description

Axen™ Plant DNA mini Kit is designed for easy and fast purification of total DNA from various plant tissue samples including leaves, stems, or roots. Secondary metabolites of plants, such as polysaccharides and polyphenols, are easily removed during the optimized procedure, and it takes just 40 minutes. Purified DNA is free of contaminants and enzyme inhibitors and is highly suited for downstream applications such as PCR, blotting, genotyping and various enzymatic reactions.

Kit contents

Contents*			Storage conditions
Cat. No.	**MG-P-008-50**	**MG-P-008-200**	
No. of preparation	50	200	
Spin column HD with tubes	50 ea	200 ea	Room Temperature
PreSep™ Column	50 ea	200 ea	
Buffer PA1	30 mL	110 mL	
Buffer PA2	12 mL	40 mL	
Buffer PA3 (conc.)**	17 mL	60 mL	
Buffer PW (conc.)**	13 mL	48 mL	
Buffer EA***	15 mL	50 mL	
RNase A solution (100 mg/mL)****	0.22 mL	0.85 mL	4˚C

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

** Buffer PA3 and PW are provided as concentrates. Absolute ethanol (ACS grade or better) must be added before first use as indicated on the bottle labels.

*** 10 mM Tris-HCl, pH 9.0, 0.5 mM EDTA

**** RNase A solution is delivered under ambient condition and can be stored at room temperature for 6 months without significant decrease in activity. But for prolonged conservation of activity, it is recommended to store at 2 ~ 8˚C.

Product use limitations

Axen™ Plant DNA 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 DNA 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 PA3 contains chaotropes which can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Preparation of ethanol-added buffer

Buffer PA3 and PW are provided as concentrate. Absolute ethanol (ACS grade or better) should be added as below before first use.

Buffer name	Included in	Volume of contents	**Ethanol to be added**	The final volume
PA3	MG-P-008-50	17 mL	**34 mL**	51 mL
	MG-P-008-200	60 mL	**120 mL**	180 mL
PW	MG-P-008-50	13 mL	**52 mL**	65 mL
	MG-P-008-200	48 mL	**192 mL**	240 mL

Protocol for preparation of plant DNA

To check before start

- Prepare water bath or dry bath at 65°C.
- Required materials; 1.5 mL and 2.0 mL centrifuge tube.
- All centrifugation steps should be performed at full speed (>10,000 xg, 10,000 ~ 14,000 rpm) in a microcentrifuge at room temperature.
- Do not use the precipitated buffers. If a precipitate forms in any buffer, dissolve completely at 20 ~ 40°C before use.

!!! Before first use, add the indicated volume of absolute ethanol to buffer PA3 and PW. (Refer the *previous page*)

1. **Place up to 100 mg (wet) or 25 mg (dried) of ground plant tissue into a 1.5 mL or 2.0 mL microcentrifuge tube.**
   - *Grind fresh or frozen plant tissue to a fine powder quickly and completely, using a mortar and pestle pre-cooled with liquid nitrogen.*
   - *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. Follow the instruction manuals for those methods.*
   - *Quick and complete pulverization at low temperature is essential for good result. But, lyophilized tissue can be ground at room temperature.*
2. **Add 400 uL of buffer PA1 and 4 uL of RNase A solution (100 mg/mL) into the tube and vortex vigorously for 15 secs.**
   - *Mix completely to make the lysate homogenate without any clumps.*
3. **Incubate for 15 mins at 65 ℃.**
   - *Occasional vortex during incubation may accelerate the lysis. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
4. **Add 135 uL of buffer PA2 to the tube and vortex vigorously for 15 secs to mix completely.**

5. **Incubate for 5 mins on ice.**

   - *The lysate of some plant tissues becomes very viscous and/or sticky after addition of buffer PA2 and this leads to clogging of PreSep™ column at next step. In this case, it is recommended to preliminarily centrifuge for 1 mins at full speed and use the supernatant for the next step.*

6. **Transfer the mixture to a PreSep™ column and centrifuge for 2 mins.**

   - *Before transfer, the mixture would be very thick and it is recommended to use a 'Wide-bore tip' for transfer or to cut off the end of pipet tip with sterile scissors.*
   - *Small pellet can be formed on the bottom of the collecting tube after centrifugation. Be careful not to disturb this pellet in next step.*

7. **Transfer the pass-through to a new 1.5 mL microcentrifuge tube by pipetting or decanting carefully without disturbing the debris pellet.**

   - *About 450 uL of the passed-through mixture would be recovered typically. It will vary depending on the plant tissue used. Check the exact volume of lysate for optimal binding condition on next step.*

8. **Add 1.5 volume of buffer PA3 to the mixture and mix thoroughly by inverting the tube upside down.**

   - *For 450 uL mixture, add 675 uL of buffer PA3.*
   - *Be careful not to vortex the tube vigorously. Intensive vortexing can shear DNA.*
   - *A precipitate can be formed in the tube after addition of buffer PA3 but this will not affect the preparation.*

9. **Transfer 750 uL of the mixture into a spin column, centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

   - *Any precipitate formed in the mixture should be transferred together.*

10. **If the mixture has remained, repeat the step 9 with them.**

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

12. **Apply 300 uL of buffer PW to the spin column, centrifuge for 2 mins. Transfer carefully the spin column into a new 1.5 mL microcentrifuge tube.**

    - *Care must be taken when removing the spin column from the collecting tube. The spin column should not come into contact with the pass-through fraction, so as this will result in carryover of ethanol.*
    - *Residual ethanol in eluate may interfere with the subsequent reactions. If carryover of wash buffer occurs, centrifuge again for 1 min before proceeding to next step.*

13. **Apply 50~100 uL of buffer EA onto the center of spin column membrane and incubate for 5 mins at room temperature. Centrifuge for 1 min for eluting the DNA.**

    - *Elution volume can be decreased to 50 uL for higher concentration of DNA, but this will slightly decrease in overall DNA yield. If maximum recovery of DNA is preferred or the starting materials contain large amount of DNA, the elution volume can be increased to 200 uL.*

Appendix.

Procedure for preparation of DNA from fungi

To check before start

- Prepare 37°C and 95°C (*optional*) waterbath or drybath.
- Prepare one of the following enzyme mixture as you need;

Lyticase mixture	Zymolyase mixture
100 unit/mL lyticase	10 unit/mL zymolyase
50 mM Tris-HCl, pH 7.5	1 M D-sorbitol
10 mM EDTA	50 mM potassium phosphate, pH 7.5
0.2%(v/v) β-mercaptoethanol	0.1%(v/v) β-mercaptoethanol

- Prepare 50 mM sodium hydroxide solution (*optional*)

1. **Harvest fungal cell pellet by centrifugation and discard the supernatant as much as possible.**
   - *20 ~ 30 ug genomic DNA will be obtained from 1~5 x 10^6 fungal cells.*
2. **(*Optional*) Add 200 uL of 50 mM sodium hydroxide solution into the tube, cover with mineral oil and incubate at 95°C for 10 mins, centrifuge at 5,000 xg for 10 mins, and discard the supernatant including mineral oil.**
   - *This optional treatment with NaOH is required only for fungi that are difficult to digest with lyticase alone, such as Aspergillus niger.*
3. **Apply 500 uL of the prepared enzyme mixture, mix gently, and incubate at 37°C for 30 mins to produce spheroplasts.**
4. **Centrifuge at full speed for 10 mins and discard the supernatant as much as possible.**
5. **Proceed to step 2 of the Plant protocol with the fungal spheroplast pellet.**

Trouble shooting guide

Facts	*Possible causes*	Recommendations
Low recovery	*Too much starting materials*	Too much starting materials may bring about inefficient lysis and spin column clogging, followed by poor DNA yields. Reduce the amount of starting material as described on procedure.
	Too old or misplaced sample used	DNA can be degraded, especially when the tissues are too old or incorrectly stored. Use fresh samples.
	Insufficient disruption/lysis	Pulverizing of the sample is critical step for good result. Improperly disrupted sample will result in poor lysis, followed by poor yield. Pulverize completely the tissue to get homogenate.
	Ethanol was not added to the buffer	Buffers PA3 and PW are provided as concentrate. Ethanol must be added to these buffers before first use. If not, the result will be significantly poor.
	Improper elution	As user's need, elution buffer other than buffer EA can be engaged. However, the conditions of optimal elution should be low salt concentration with weak alkaline pH (7<pH<9). When water or other buffer was used as eluent, ensure that condition. After eluent is applied on the center of spin column membrane, it is essential to incubate at least for 5 minutes at room temperature.
Low purity	*Incomplete precipitation*	Any cell debris or precipitates must be removed in step 7 before addition of buffer PA3.
	Insufficient lysis	Too much starting material can lead to poor lysis, followed by low purity of DNA.
Clogging of PreSep^{TM}column	*High viscosity of lysate*	Refer to the note of step 5. A preliminary centrifugation can help lower the viscosity.
Clogging of spin column	*Incomplete removal of precipitate*	Any cell debris or precipitates must be removed in step 7 before addition of buffer PA3, because these can clog the pores of spin column membrane.
	Lysate too viscous or sticky	Reduce the amount of starting sample, or increase the amount of buffers proportionally.

Degraded DNA	*Too old or misplaced sample used*	DNA can be degraded, especially when the tissues are too old or incorrectly stored. Use fresh samples.
	Excessive or retarded shredding	It is essential for good result to pulverize the sample thoroughly. However, excessive or retarded shredding of sample will lead to damage on DNA.
Enzymatic reaction is not performed well with purified DNA	*High salt concentration in eluate*	Ensure that wash step is carried out just in accordance with the protocols. Additional PW washing may help remove salts from the membrane.
	Residual ethanol in eluate	The spin column membrane should be dried completely before eluting DNA. Perform additional centrifugation to dry the membrane, if needed.