Axen™ Plant Seeds and Bulbs DNA Kit

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Product description

Axen™ Plant Seeds and Bulbs DNA Kit provides the simple and rapid method for extraction of total DNA from various plant seeds and bulbs. Purified DNA is free of contaminants and enzyme inhibitors and is highly suited for downstream applications such as PCR, genotyping and various enzymatic reactions.

Kit contents

Contents*			**Storage conditions**
Cat. No.	**MG-P-010-50**	**MG-P-010-200**	
No. of preparation	50	200	
Mini spin column	50 ea	200 ea	Room Temperature
Buffer GML	55 mL	220 mL	
Buffer GMA	55 mL	220 mL	
Buffer GMB	30 mL	110 mL	
Buffer PW1 (conc.)**	18 mL	75 mL	
Buffer PW2 (conc.)**	17 mL	34 mL x 2	
Buffer EA***	15 mL	50 mL	
Proteinase K solution (20 mg/ml)****	0.55 mL	1.05 mL x 2	4˚C

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

** Buffer PW1 and PW2 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

**** Proteinase K should be stored under 4°C on arrival for conservation of activity. Delivery time would not lead to noticeable loss of the enzyme's activity. Proteinase K solution can be stored at 4°C for a year without significant decrease of performance.

Product use limitations

Axen™ Plant Seeds and Bulbs DNA 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 Seeds and Bulbs DNA 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 GMB and PW1 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.

Required Materials Not Provided

Reagents	- 70% ethanol, ACS grade or better - Absolute ethanol, ACS grade or better
Disposable materials	- Sterile 1.5 or 2.0 mL microcentrifuge tubes and pipette tips
Equipments	- Equipment for disrupting samples, - Devices for liquid handling - Heat block or water bath - Microcentrifuge - Suitable protector (ex; lab coat, disposable gloves, goggles, etc)

Preparation of ethanol-added buffer

Buffer PW1 and PW2 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
PW1	MG-P-010-50	18 mL	**18 mL**	36 mL
	MG-P-010-200	75 mL	**75 mL**	150 mL
PW2	MG-P-010-50	17 mL	**68 mL**	85 mL
	MG-P-010-200	34 mL x 2	**136 mL x 2**	170 mL x 2

Procedure for preparation of DNA

❖ We recommend you read this protocol thoroughly before start.

Prepare water bath or dry bath at 65°C.

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°C ~ 40°C before use.

1. Place the ground sample into a 1.5 mL or 2.0 mL microcentrifuge tube according to the below table.

	Recommended amount	Maximum amount
Seeds	100 mg	200 mg*
Bulbs	200 mg	500 mg**

* When the sample is Alfalfa or Flax, the maximum limit is 100 mg.

** Do not exceed 100 mg per prep when dried bulb sample is used.

➢ *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. Also, other methods like a bead-beating instrument or a rotor-stator homogenizer can be good alternatives. Follow the instruction manuals for those methods.*

2. Add 10 uL of proteinase K solution and 1 mL of the appropriate lysis buffer according to below table.

Lysis buffer	Seeds	Bulbs
GML	Bean, Carnation, Chicory, Eggplant, Flax, Hot pepper, Lentil, Maize, Melon, Petunia, Rice, Safflower, Squash, Sugar beet, Sugarcane, Sweet pepper, Tomato	Allium, Anemone, Buttercup, Chinese peony, Copperlily, Ginger, Tulip, Wood sorrel
GMA	Alfalfa, Canola, Cotton, Creeping bentgrass, Soybean, Wheat	Belladonna lily, Blazing star, Calla, Crocus, Dahlia, Garlic, Grape hyacinth, Hyacinth, Iris, Lily, Paperwhite, Red spider lily, Shallot, Sword lily

3. **Vortex vigorously for 15 secs and incubate for 30 mins at 65℃.**
   - *Mix completely to make the lysate homogenate without any clumps.*
   - *Occasional vortex during incubation may accelerate the lysis. Use of a specialized instrument such as thermo-mixer will accelerate the lysis.*
4. **Centrifuge for 5 mins and transfer 500 uL of supernatant into a new 1.5 mL microcentrifuge tube.**
   - *When the sample contains high-lipid contents, a lipid film can be formed on the top layer after centrifugation. In this case, take the middle clear-layer carefully by pipet.*
   - *Be careful not to co-transfer the debris as much as possible.*
5. **Apply an equal volume (500 uL) of buffer GMB into the tube**
6. **If the applied sample corresponds to the items below, add 200 uL of isopropanol to the tube.**

Seeds	Bulbs
Carnation, Chicory, Creeping bentgrass, Eggplant, Maize, Petunia, Rice, Safflower, Sugar beet, Sugarcane, Sweet pepper, Wheat	Belladonna Lily, Blazing star, Calla, Copperlily, Crocus, Dahlia, Garlic, Ginger, Grape hyacinth, Hyacinth, Iris, Lily, Paperwhite, Red spider lily, Shallot, Sword lily, Tulip, Wood sorrel

7. **Mix well by vortexing for 10 secs**
   - *Do not centrifuge the tube after addition of isopropanol.*
8. **Transfer 750 uL of the mixture into a spin column, centrifuge for 30 secs, discard the passed-through, and re-insert the column back into the tube.**
   - *Be careful not to co-transfer the debris. It can cause clogging of spin column.*
9. **Repeat step 8 with the remaining sample.**
10. **Apply 650 ul of buffer PW1 to the spin column, centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

11. **Apply 750 ul of buffer PW2 to the spin column, centrifuge for 30 secs, empty the collecting tube and re-insert the spin column back to the tube.**

12. **Repeat step 11 once.**

13. **Centrifuge the tube for 1 min for drying the membrane and transfer the spin column into a new 1.5 mL microcentrifuge tube.**

    - *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.*

14. **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 30 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 maximum.*

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 PW1 and PW2 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	*Contamination of debris*	Any cell debris or precipitates must be excluded when transferring the supernatant at step 4
	Too low yield	When the yield is very low, the absorbance may not represent properly its purity.
	Insufficient lysis	Too much starting material can lead to poor lysis, followed by low purity of DNA.
Clogging of spin column	*Incomplete removal of precipitate*	Any cell debris or precipitates must be excluded when transferring the supernatant at step 4, 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.
	RNase treatment needed	Sometimes degraded RNA can be confused with DNA smearing on electrophoresis. RNA can be removed by treatment of RNase A.
	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 PW2-washing can 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.


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