Axen™ Plasmid DNA miniprep Kit

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Product description

Axen™ Plasmid miniprep kit provides the fast and simple method for the mini scale preparation of plasmid DNA from bacterial cells. This kit utilizes the spin column and silica-binding technology, and is ready for the preparation of plasmid DNA from *endA*$^{+}$ strains. Plasmid DNA can be prepared in less than 25 minutes without any use of hazardous organic solvents and alcohol precipitation, and this purified DNA can be directly used in various downstream applications, such as PCR, cloning, sequencing, transfection and enzymatic reactions, without any further manipulations.

Kit contents

Contents*			Storage conditions
Cat. No.	**MG-P-001-50**	**MG-P-001-200**	
No. of preparations	50	200	Room Temperature
Mini column and Collecting tube	50 ea	200 ea	
Buffer P1**	15 mL	55 mL	
Buffer P2***	15 mL	55 mL	
Buffer P3***	20 mL	75 mL	
Buffer EW (Concentrates***,****)	15 mL	55 mL	
Buffer PW (Concentrates****)	10 mL	30 mL	
Buffer EB*****	20 mL	50 mL	
RNase A solution** (20 mg/mL)	75 uL	275 uL	4˚C

* 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.

** Before first use, add all of RNase A solution into buffer P1 and mix gently. Buffer P1 should be stored at 2~8˚C after addition of RNase A.

*** During shipment or storage under cold ambient condition, a precipitate can be formed in buffer P2 and/or P3. Heat the bottle at 20~40˚C to dissolve completely in such a case.

**** Buffer EW and buffer PW are provided as concentrates. Ethanol must be added before first use as indicated on the bottle labels.

***** 10 mM Tris-HCl, pH 8.5

Product use limitations

Axen™ Plasmid DNA miniprep 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™ Plasmid DNA miniprep 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 P3 and buffer EW 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.

Protocol for preparation of plasmid DNA

To check before start

- Required consumables; 1.5 mL centrifuge tube
- Do not use the precipitated buffers. If a precipitate forms in a buffer, dissolve completely at 20~40°C before use.

Before first use, do the followings;

① Add *absolute* ethanol into the wash buffers as below.

Buffer name	Included in	Volume of contents	**Ethanol to be added**	The final volume
EW	MG-P-001-50	15 mL	**15 mL**	30 mL
	MG-P-001-200	55 mL	**55 mL**	110 mL
PW	MG-P-001-50	10 mL	**40 mL**	50 mL
	MG-P-001-200	30 mL	**120 mL**	150 mL

② Add all of RNase A solution into the bottle of Buffer P1 and mix well by gentle swirling. Store the buffer P1 at 2~8°C after addition of RNase A.

1. **Harvest up to 5 mL of bacterial culture by centrifugation for 5 mins at 10,000 xg. Discard the supernatant as much as possible without disturbing the pellet.**
   - *Alternatively, bacterial cells can be pelleted repeatedly in 1.5 mL or 2 mL microcentrifuge tube, by centrifugation for 1 min at full speed*
2. **Resuspend the pelleted cells thoroughly in 250 uL of buffer P1. Transfer the suspension to a new 1.5 mL microcentrifuge tube.**
   - *It is essential to thoroughly resuspend the cell pellet.*
3. **Add 250 uL of buffer P2 and mix gently by inverting until evenly blue. Let it stand until the mixture becomes transparent.**
   - *Do NOT vortex at this step and do NOT incubate the mixture for more than 5 mins.*
4. **Add 350 uL of buffer P3 and mix immediately but gently by inverting until the mixture returns to evenly clear again.**

5. **Centrifuge for 10 mins at 13,000 xg.**

6. **Transfer carefully the supernatant to a spin column and centrifuge for 30 secs at 13,000 xg. Remove the spin column, discard the pass-through, and insert the column back into the collection tube.**
   - *Avoid the white precipitate co-transferring into the SV column.*

7. **(*Optional*) Apply 500 uL of buffer EW into the column and centrifuge for 30 secs at 13,000 xg. Remove the spin column, discard the pass-through, and insert the column back into the collection tube.**
   - *This step is necessary to remove any traces of nuclease activity from endA+ strain.*
   - *When low-copy plasmid is used, it is recommended to carry out this step, even though endA- strains.*

8. **Apply 700 uL of buffer PW into the column and centrifuge for 30 secs at 13,000 xg. Remove the spin column, discard the pass-through, and insert the column back into the collection tube.**

9. **Centrifuge for 1 min at 13,000xg for drying the membrane and transfer the spin column into a new 1.5 mL tube.**
   - *This step removes residual ethanol from spin column membrane. Residual ethanol in eluate may inhibit subsequent enzymatic reaction. If carryover of buffer PW occurs, centrifuge again for 1 min before proceeding to next step.*

10. **Apply 50 uL of buffer EB or nuclease-free water to the center of spin column membrane and let it stand for 1 min.**
    - *Ensure that buffer EB or nuclease-free water is dispensed directly onto the center of spin column membrane for optimal elution of DNA.*
    - *For long-term storage, eluting in buffer EB (10 mM TrisCl, pH 8.5) and storing below -20℃ is recommended. When using water for elution, ensure that the pH of water is within the range of 7.0~8.5.*

11. **Centrifuge at 13,000 xg for 1 min for eluting the DNA.**

Trouble shooting guide

Facts	*Possible causes*	Recommendations
Low recovery of plasmid DNA	*Too many bacterial cells in the sample*	Overloaded sample cannot be lysed efficiently, followed by poor yield of plasmid DNA. Bacterial cultures should be grown for 12~21 hours in proper media with antibiotics. Reduce the starting sample especially when rich broth is used, such as TB or 2xYT.
	Low copy-number plasmid used	Increase the starting sample with an additional buffer for low-copy number plasmid.
	Poorly resuspended bacterial pellets	Bacterial pellets should be re-suspended thoroughly in buffer P1. The clump of cells cannot be lysed efficiently.
	Buffer P2 precipitated	Make sure the buffer P2 not to have any precipitates before use. Precipitated buffer P2 will lead to poor lysis of bacterial cells.
	RNA not digested	Excessive RNA can interfere with the binding of plasmid to a column membrane. The buffer P1 containing RNase A should be stored at 2~8℃ for the conservation of activity.
	Improper elution	As user's need, elution buffer other than buffer EB 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, make sure that condition.
Low purity	*Precipitates contaminated*	Any cell debris or precipitates should not be co-transferred when applying the supernatant to the spin column at step 6.

Genomic DNA contaminated	*Excessive handling of lysate after adding the Buffer P3*	Excessive or vigorous impact to the lysate may lead precipitated genomic DNA to be extruded into the solution. The P3-added lysate should be handled carefully before centrifugation.
Plasmid DNA smeared	*Excessive handling of lysate after adding the Buffer P2*	Excessive lysis time and/or vigorous vortexing of the lysate after addition of buffer P2 will lead to irreversible denaturation of plasmid DNA. The lysis time should not over 5 mins and the lysate should be handled carefully.
Plasmid DNA degraded	*Endonuclease not removed completely*	Perform step 7 for *endA*+ strains such as HB101 and JM series. Endonuclease will be washed away by EW-washing.
RNA contaminated	*RNase A omitted or inactive*	All of RNase A solution should be added to buffer P1 before first use. Buffer P1 containing RNase A should be stored at 2~8℃. Add an additional RNase A (final conc. 100 ug/mL) when the activity is decreased.
Enzymatic reaction is not performed well with purified DNA	*High salt concentration in eluate*	Ensure that wash step was 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.


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