Axen™ Gel DNA Kit

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Product description

Axen™ Gel DNA Kit provides simple and fast method for extraction of DNA from agarose gel. DNA of 80 bp to 10kb in length can be recovered from standard or low-melting agarose gel made from TAE or TBE. Purified DNA can be directly used in ligation, labeling, sequencing, PCR, and many other downstream applications without further manipulation.

Kit contents

Contents*			Storage conditions
Cat. No.	**MG-P-003-50**	**MG-P-003-200**	
No. of preparation	50	200	
Mini column and Collecting tube	50 ea	200 ea	Room Temperature
Buffer GB**	50 mL	200 mL	
Buffer NW (Concentrates***)	10 mL	40 mL	
Buffer EB****	15 mL	30 mL	

* 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 GB. Heat the bottle at 20~40°C to dissolve completely in such a case.

*** Buffer NW is provided as concentrates. Ethanol must be added before first use as the indication on the bottle label.

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

Product use limitations

Axen™ Gel 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™ Gel 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 GB 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.

Procedure for extraction of DNA from agarose gel

To check before start

- Prepare water bath or dry bath at 50˚C
- 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, add the indicated volume of absolute ethanol to buffer NW as below table.

Buffer name	Included in	Volume of Contents	**Ethanol to be added**	The final volume
NW	MG-P-003-50	10 mL	**40 mL**	50 mL
	MG-P-003-200	40 mL	**160 mL**	200 mL

1. **Excise the DNA band of interest using a sterile razor blade or scalpel on a transilluminator.**
   - *Minimize a gel volume by cutting the gel slice as small as possible.*
   - *Do cutting the gel rapidly on a long wave length-UV. Long exposure to UV rays can damage DNA, followed by failure of enzymatic reactions.*
2. **Weigh the gel slice in a microcentrifuge tube and add 3 volumes (uL) of buffer GB into the tube per 1 volume(mg) of sliced gel.**
   - *Apply 300 uL of buffer GB for 100 mg of sliced gel.*
3. **Incubate at 50°C until the agarose gel is completely melted.**
   - *A sliced gel is generally melted completely in 10 mins.*
   - *To accelerate melting of gel, vortex the tube every 2~3 mins during the incubation.*
4. **Briefly vortex the tube for homogenization of the mixture and check that the color of the mixture is still yellow.**
   - *If the color has been changed to brown or purple, add 10 uL of 3M sodium acetate, pH 5.0, and mix well for adjusting of binding pH. Ignore the change of color due to loading dye.*

5. **(*Optional:*) Add 1 gel volume of isopropanol to the sample and vortex thoroughly to mix.**
   - *For 100 mg of gel slice, apply 100 uL of isopropanol.*
   - *Do NOT centrifuge after the addition of isopropanol.*
   - *This step should be carried out when the size of the target is shorter than 200 bp or longer than 5 kb. It will hardly have effect for the size between 200 bp and 5 kb.*
6. **Transfer 700 uL of the mixture into a spin column and centrifuge for 30 secs at 13,000xg.**
7. **Remove the spin column, discard the pass-through, and insert the column back into the collection tube.**
   - *If the mixtures are remained, repeat the step 6 ~ 7 with them.*
8. **Apply 700 uL of buffer NW into the column and centrifuge for 30 secs at 13,000 xg.**
   - *If the purified DNA should be used for a salt-sensitive experiment, it is recommended to let the spin column stand for 5 mins after the addition of buffer NW, making some amount of the buffer flow through the membrane by gravity.*
9. **Remove the spin column, discard the passed-through, and insert the column back into the collection tube.**
10. **Centrifuge for 1 min at 13,000xg or full speed for drying the membrane and transfer the spin column into a new 1.5 mL tube.**
    - *If a residual buffer remains in the spin column, centrifuge again for an additional 1 min at full speed before transferring to a new 1.5 mL tube. Residual ethanol may inhibit the subsequent enzymatic reactions.*
11. **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 the buffer EB or nuclease-free water is dispensed directly onto the center of spin column membrane for optimal elution of DNA.*
    - *The eluent volume can be increased to 200 uL for maximum recovery, but it will decrease the concentration of DNA. The eluent volume should not be lower than 30 uL, because the smaller volume cannot soak the membrane entirely.*
12. **Centrifuge at 13,000 xg for 1 min for eluting the DNA.**
    - *Store the DNA eluate under -20℃ for long-term storage.*

Appendix.

Procedure for purification of PCR amplicon

- *This procedure provides the method of purifying DNA from the enzymatic reactions such as PCR.*

1. **Add 5 volumes of buffer GB for 1 volume of PCR amplicon and vortex to mix completely.**
   - *For example, apply 500 uL of buffer GB for 100 uL of the amplicon volume.*
2. **(*Optional:*) Add 2 PCR amplicon volume of isopropanol to the mixture and vortex thoroughly to mix.**
   - *For 100 uL of the PCR amplicon, apply 200 uL of isopropanol.*
   - *Do NOT centrifuge after the addition of isopropanol.*
   - *This step can be applied when the size of the target is longer than 5 kb. Note that this step can lead to co-purification of primer-dimer.*
3. **Transfer up to 700 uL of the mixture to a spin column.**
4. **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.**
   - *If the mixtures are remained, repeat the step 3 ~ 4 with them.*
5. **Apply 700 uL of buffer NW into the column and centrifuge for 30 secs at 13,000 xg.**
   - *Make sure that the ethanol is added to buffer NW before first use. Refer to the previous protocol.*
6. **Remove the spin column, discard the pass-through, and insert the column back into the collection tube.**
7. **Centrifuge for 1 min at 13,000xg or full speed for drying the membrane and transfer the spin column into a new 1.5 mL tube.**
8. **Apply 50 uL of buffer EB or sterile water to the center of spin column membrane and let it stand for 1 min.**
   - *Ensure that the buffer EB or nuclease-free water is dispensed directly onto the center of spin column membrane for optimal elution of DNA.*
   - *The eluent volume can be increased to 200 uL for maximum recovery, but it will decrease the concentration of DNA. The eluent volume should not be lower than 30 uL, because the smaller volume cannot soak the membrane entirely.*
9. **Centrifuge at 13,000 xg for 1 min for eluting the DNA.**

Trouble shooting guide

Facts	*Possible causes*	Recommendations
Low or no recovery	*Incompletely solubilized gel*	The sliced agarose gel should be completely dissolved without any particles. To assist the complete solubilization, mix the tube by vortexing every 2~3 minutes during incubation or increase the incubation time. To use low melt agarose usually results in better recovery.
	Too high pH of binding mixture	At high pH, the binding of DNA to silica membrane will be significantly reduced. The dye included in buffer GB indicates the pH of mixture as color change from yellow at optimal pH to brown or purple at abnormally higher pH. If the color of mixture has turned to brown or purple, add 10 µl of 3 M sodium acetate,pH 5.0 to the sample and mix well. The color of mixture will turn to yellow indicating the correct pH for DNA binding.
	Improper eluent	An eluent other than buffer EB can be employed as user's needs. The optimal eluting condition should be under low salt concentration with alkaline pH (7.0<pH<9.0). When water or other buffer was used as eluent, ensure that condition.
	Ethanol was not added to the buffer	Buffer NW is provided as concentrate. Ethanol must be added to these buffers before first use. Otherwise, the result will be significantly poor.
Ligation failure	*Too long or strong exposure to UV on transilluminator*	UV destroys the DNA ends. Use UV of longwave length and make the handling time as short as possible when excising the gel slice.
Clogging of spin column	*Incompletely solubilized gel*	See the section of 'Low or no recovery'
	High percentage-agarose gel used	For >1.5% agarose gel, 5 volumes of buffer GB to 1 volume of gel slice should be added. For 100 mg of agarose gel, add 500 µl of buffer GB. If the mini column is clogged, transfer the mixture from the mini column to a 1.5 ml microcentrifuge tube, add 1 volume of buffer GB to mixture volume. Incubate for 5 minutes at 50˚C, proceed again to binding steps.
Non-specific band appears	*DNA denatured*	Re-nature the DNA by heating up to 95˚C for 1 minute and let cool slowly to room temperature.
Enzymatic reaction isn't performed well with purified DNA	*High salt concentration in eluate*	Ensure that wash step is carried out just in accordance with the protocols. Additional wash step may help to remove high salt in eluate.
	Residual ethanol in eluate	Ensure that the wash step on the protocol is performed properly. The spin column membrane should be dried completely before eluting DNA. Perform an additional centrifugation to dry the membrane, if needed.


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