Gel DNA Recovery Kit

REF EX6151 Σ 25 Tests

RUO

15°C 25°C

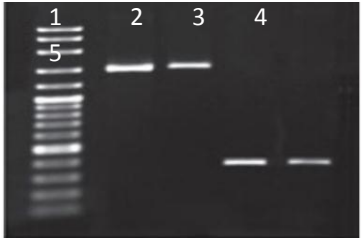
Components

Contents	Quantity/ Volume
Binding Buffer	30ml
Washing Buffer*	4ml
Elution Buffer	2×1250µl
Mini column	25PCS

***Do not forget to add the appropriate amount of Ethanol (96–100%) to Washing Buffer as indicated on the bottle, before using for the first time.**

Description

Gel DNA Recovery Kit provides reliable and fast method for an efficient isolation of 80bp to 10kb fragments from standard or low-melting agarose gel in TAE or TBE buffer system. Typical yields of this kit are almost 80%. Purified DNA can be directly used in ligation, labelling, sequencing and many other downstream applications without further manipulation.



1. DNA Ladder
2. DNA fragment 1.5Kb
3. Recovered DNA fragment 1.5Kb
4. DNA fragment 300bp
5. Recovered DNA fragment 300bp

Required materials that are not supplied with the kit

1. Absolute ethanol
2. Isopropanol
3. 3M sodium acetate, pH 5.0

Important notes: please read before starting

- Binding buffer contains chaotropic salt, which is irritant. Take appropriate laboratory safety measures and wear gloves when handling. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.
- Usually low melt agarose gel results in a better recovery yield. The components and pH of starting sample can alter the pH of the mixture with binding buffer, especially in extraction of DNA from agarose gel. Binding buffer contains pH indicator in order to check this alteration of binding condition. If the color (around yellow) of binding mixture turns to red after addition of Binding buffer, it means that the pH of binding mixtures is higher than the optimal.

The indicator dye is completely removed during subsequent washing step and does not interfere the downstream applications.

- Before first use, add absolute ethanol to Washing Buffer as indicated on the bottle. (Add 16ml absolute ethanol in Washing Buffer before use)
- All centrifugation should be carried out above 10,000g (>12,000rpm) at room temperature.
- Prepare water bath or heating block to 55˚C.
- For large fragments (>5kb), pre-warm Elution Buffer to 70˚C.
- During shipment or storage under cool ambient condition, a precipitate can form in Binding buffer. In such a case, heat the bottle to 50˚C to dissolve completely.

Protocol

1. Excise the DNA band of interest using an ethanol-cleaned razor blade or scalpel on a transilluminator. Standard sample size is 200mg of gel (400mg max). Minimize gel volume by cutting the gel slice as small as possible.
2. Weigh the gel slice in a microcentrifuge tube. Add 3 volumes (μl) of Binding buffer to 1 volume (mg) of gel.
   For example, add 300μl of Binding buffer to each 100mg of agarose gel slice. For >1.5% agarose gel, add 5 volumes of Binding buffer.
3. Incubate at 55°C until the agarose gel is completely melted (5~10min). To help the efficient dissolving of gel, vortex the tube every 2~3min during the incubation.
4. After the slice has dissolved completely, check that the color of the mixture is yellow (similar to Binding buffer) *[1].

*[1]If the color of the mixture becomes red or purple, add 10μl of 3M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow. Adjusting of pH is not needed.

5. Add 1 gel volume of isopropanol to the sample and vortex to mix. For 100 mg of gel volume, add 100μl of isopropanol.
6. Transfer the mixture to a Column. Centrifuge for 2min above 10,000g (>12,000 rpm). Discard the pass-through and re-insert the mini column into the collection tube *[2].

*[2]If the mixture volume is larger than 700μl, apply the mixture twice; apply 700μl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat the step again until all of the mixture has been applied to the mini column.

7. Add 700μl of Washing Buffer to the mini column. Centrifuge for 2min above 10,000g (>12,000rpm). Discard the pass-through and re-insert the mini column into the collection tube.
8. Centrifuge for an additional 2min at full speed to remove residual wash buffer. Transfer the mini column to a new 1.5ml microcentrifuge tube *[3].
9. Apply 30-50μl of Elution Buffer or ddH2O to the center of the membrane in the mini column, let stand for 5-10min in 55˚C and centrifuge for 2min above 10,000g (>12,000rpm) *[4].

*[3]For higher overall yield, increase the volume of Elution Buffer and repeat the elution step once again. Optimal results may be obtained by eluting twice. More 20~40% DNA can be acquired by repeat of eluting once again. A new 1.5ml tube can be used to prevent dilution of the first eluate.

*[4]The minimum elution volume is 30μl and lower volume will decrease the yield significantly. Up to 200μl of Elution Buffer can be applied and it results in low concentration of DNA. Higher concentrated DNA will be obtained with lower elution volume.

Troubleshooting

This guide may help to solve problems that may arise.

Facts	Possible Causes	Suggestions
Low or no recovery	Incompletely solubilized gel	- Increase the incubation time. - Use low melt agarose. - For >1.5% agarose gel, add 5 volumes of Binding buffer to 1 volume of gel slice.
	Too high pH of binding mixture	- Add 10µl of 3M sodium acetate, (pH 5.0) to the sample and mix
	Improper Elution Buffer	- As user's requirement, elution buffer can be replaced with any eluent. The condition of optimal elution is low salt concentration with alkaline pH (7.0<pH<9.5). When water or other buffer was used as eluent, ensure that conditions.
	Elution Buffer incorrectly dispensed	- Ensure that Elution Buffer dispensed to the center of membrane. Incorrectly dispensed Elution Buffer causes inappropriate contact with membrane, followed by poor DNA recovery.
Ligation failure	Too long or strong exposure to UV on transilluminator	- UV destroys the DNA ends. Use UV of long wave length and make the handling time as short as possible when excising the gel slice.
Clogged membrane	Incompletely solubilized gel	- See the section 'Incompletely solubilized gel' in the Facts "Low or no recovery" - transfer the mixture from the mini column to a 1.5ml microtube, add 1 volume of Binding buffer to mixture volume. Incubate for 5 minutes at 55˚C, proceed again to step 4.

Facts	Possible Causes	Suggestions
Enzymatic reaction is not performed well with the purified DNA	Residual ethanol from Wash Buffer remains in eluate	- Centrifuge column again for an additional 1min at full speed before transferring to a new 1.5ml microcentrifuge tube. Residual ethanol from Wash Buffer can inhibit subsequent enzymatic reaction.
	Too high salt concentration in eluate	- Let the mini column stand for 5min after addition of Washing Buffer, making some amount of wash buffer flow through the column by gravity before centrifugation. - Repeat the washing step.
	Eluate contains denatured ssDNA	- For reannealing of ssDNA to dsDNA, incubate ssDNA at 95˚C for 2 minutes, and then allow to cool slowly at room temperature
DNA floats out while loading on agarose gel	Residual ethanol from Washing Buffer remains in eluate	- Centrifuge column again for complete removal of ethanol at step 8.
Non-specific band appears after purification	DNA denatured	- Renature the DNA by warming up to 95˚C for 1 minute and let cool slowly at room temperature

Signs

Signs	Definitions
	Temperature range on product use
RUO	For Research Use Only
	Product shipping conditions
	Name and address of the manufacturer of the product
REF	Product technical code

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