

PCR Clean Up Kit

REF EX6141

Σ 50 Tests

RUO

15°C 25°C

Components

Contents	Quantity/ Volume
Binding Buffer	25ml
Washing Buffer*	7ml
Elution Buffer	5ml
Column (mini)	50

* **Before first use, add absolute ethanol into Washing Buffer as indicated on the bottle (add 28ml absolute ethanol in Washing Buffer before use)**

Description

PCR Clean up Kit provides a simple and rapid method to purify PCR products or other enzymatic reactions in just 20 minutes. Pure DNA (100bp~10kb) can be obtained, and this purified DNA can be directly used in cloning, sequencing and many other applications. PCR Clean up Kit procedures remove the DNA fragments smaller than 100bp (primers and primer dimers in PCR products), any unwanted oligoes and impurities, such as salts, proteins, nucleotides, dyes and detergents.

Required materials that are not supplied with the kit

- Absolute ethanol

Important notes: please read before starting Washing

- 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.
- Before first use, add absolute ethanol to Washing Buffer as indicated on the bottle. (Add 28ml absolute ethanol in Washing Buffer before use.)
- All centrifugation should be carried out above 10,000g (>12,000rpm) at room temperature.
- All solutions should be equilibrated at room temperature before procedures.
- For large fragments (>5kb), pre-warm Elution Buffer to 70°C.
- Prepare water bath or heating block to 55°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. Add 5 volumes of Binding Buffer to 1 volume of the sample and mix.
For example, add 500μl of Binding Buffer to each 100μl of sample. It is not necessary to remove mineral oil.
2. Transfer the mixture to the mini-Column. Centrifuge for 2min above 10000g (>12,000rpm). Discard the pass-through and re-insert the mini column back into the collection tube.
3. Add 700μl of Washing Buffer to the mini-Column and centrifuge for 2min above 10000g (>12,000rpm). Discard the pass-through and re-insert the mini column back into the collection tube.
* If the purified DNA will be used for salt sensitive applications, 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.
4. Centrifuge for an additional 2min at full speed to remove residual wash buffer. Then Transfer the mini column to a new 1.5ml microcentrifuge tube.
* If the mini column has Washing Buffer associated with it, centrifuge again for additional 1min at full speed before transferring to the new 1.5 ml microcentrifuge tube. Residual ethanol from Washing Buffer can inhibit subsequent enzymatic reaction.

5. Add 30µl 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 10000g (>12,000rpm).

*Ensure that the Elution Buffer or distilled water is dispensed directly onto the center of column membrane for optimal elution of DNA.

*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.

*The minimum elution volume is 30µl and lower volume will decrease the yield significantly.

Troubleshooting






This guide may help to solve problems that may arise.

Facts	Possible Causes	Suggestions
Low or no recovery	Improper elution Buffer	- As user's requirement, Elution buffer other than Elution Buffer can be used. 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 conditions.
	Elution buffer incorrectly dispensed	- Ensure that Elution Buffer dispensed to the center of membrane. Incorrectly dispense Elution Buffer causes inappropriate contact with membrane, followed by poor DNA recovery.
Enzymatic reaction is not performed well with the purified DNA	Residual ethanol From Washing 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.

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Facts	Possible Causes	Suggestions
DNA floats out while loading on agarose gel	Residual ethanol From Washing Buffer remains in eluate	Centrifuge column again for an additional 1min at full speed before transferring to a new 1.5ml microcentrifuge tube.
Non-specific band appears after purification	DNA denatured	Renature the DNA by warming up to 95°C for 1 minute and let cool slowly to room temperature.

Signs

Signs	Definitions	Signs	Definitions
	Temperature range on product use		Name and address of the manufacturer of the product
	For Research Use Only		Product technical code
	Number of usable tests	-	-



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