FISHER MOLECULAR BIOLOGY

PCR Clean-up Mini Kit

DE-017 – DE-018

PCR Clean-up Mini Kit provides spin columns, buffers, and collection tubes for silica-membrane-based purification of PCR products >100 bp. DNA of up to 10 kb is purified using a simple and fast bind-wash-elute procedure and an elution volume of 40 µl.

- For purification of PCR products or reaction mixtures (concentration and desalination of reaction mixtures)

Kit components	DE-017 (50 preps)	DE-018 (300 preps)
FSDF Buffer*	40 ml	240 ml
Wash Buffer (concentrate)**	12,5 ml	50 ml
Elution Buffer	5 ml	30 ml
FSDF Column	50 pcs	300 pcs
Collection Tube	50 pcs	300 pcs
Elution Tube	50 pcs	300 pcs
User Manual	1	1
Preparation of Wash buffer adding Ethanol 96- 100%		
Ethanol volume for wash buffer**	50 ml	200 ml

*FSDF Buffer contains pH indicator allowing easy determination of the optimal pH for DNA binding to the silica membrane

Specification:

Principle: spin column (silica matrix)

DNA Binding capacity of spin column: up to 20 µg

Sample size: up to 100 µl of reaction solution

Recovery: 95% for PCR clean-up

Operation time: 10 ~ 20 min

Elution volume: 40 µl

Important Notes:

1. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffer.
2. Add the required volume of ethanol (96~100%) to Wash Buffer before use.
3. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000 ~1,8000 x g.

11. Centrifuge at full speed (~ 18,000 x g) for 1 min to elute the DNA.

PCR Clean-Up Protocol:
Please Read Important Notes Before Starting Following Steps

1. **Transfer up to 100 µl of PCR product (excluding oil) to a microcentrifuge tube (not provided) and add 5 volumes of FSDF Buffer, mix well by vortexing.**
   - For example, Add 250 µl of FSDF Buffer to 50 µl of PCR product.
   - The maximum volume of PCR product is 100 µl (excluding oil). Do not excess this limit. If PCR product is more than 100 µl, separate it into multiple tubes.
2. **Place a FSDF column into a Collection Tube.**
3. **Transfer the sample mixture to the FSDF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.**
4. **Add 750 µl of Wash Buffer (ethanol added) to the FSDF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.**
   - Make sure that ethanol (96-100 %) has been added into Wash Buffer when first open.
5. **Centrifuge again at full speed (~18,000 x g) for an additional 3 minutes to dry the column matrix.**
   - **Important step !** The residual liquid should be removed thoroughly on this step.
6. **Place the FSDF Column to a new microcentrifuge Elution tube (provided).**
7. **Add 40 µl of Elution Buffer or ddH2O to the membrane center of the FSDF Column. Stand the FSDF Column for 1 min.**
   - **Important step !** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
   - **Important :** Do not elute the DNA using less than suggested volume (40 µl). It will lower the final yield.
8. Centrifuge at full speed (~18,000 x g) for 1 min to elute the DNA.

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Troubleshooting

(For PCR Clean-Up)

Problems	Possible reasons	Solutions
Low or none recovery of DNA fragment	Apply more than 100 µl of PCR product	If PCR product is more than 100 µl, separate it into multiple tubes.
	Elution of DNA fragment is not efficient	Make sure the pH of Elution Buffer or ddH2O is between 7.0- 8.5.
		Make sure that the elution solution has been completely absorbed by the column membrane before centrifugation.
	The size of DNA fragment is larger than 5 Kb	Preheat the elution solution to 60 °C before use.
Poor perfor mance in the downstream applications	Salt residue remains in eluted DNA	Wash the column twice with Wash Buffer.
	Ethanol residue remains in eluted DNA	Do discard the flow-through after washing with Wash Buffer and centrifuge for an additional 3 min.

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