WelPrep™ PCR Purification Kit

Catalog Number **PR 005-02**
Storage Temperature **Room Temperature**

Product Description
WelPrep™ PCR Prufication Kit is optimized for efficient recovery of DNA and removal of contaminants. DNA adsorbs to the silica-membrane in the presence of high salt while contaminants pass through the column. Impurities are efficiently washed away, and the pure DNA is eluted with Elution Buffer or Water. The PCR Purification spin columns offer two handling options – as an alternative to processing the spin columns in a microcentrifuge, they can now also be used on any commercial vacuum manifold with luer connectors.

Adsorption to PCR Purification membrane
The PCR Purification membrane is uniquely adapted to isolate DNA from aqueous solutions, and up to 10 ug DNA can bind to PCR Purification column.

Washing
During the DNA adsorption step, unwanted primers and impurities, such as salts, enzymes, unincorporated nucleotides, agarose, dyes, ethidium bromide, oils, and detergents (e.g., DMSO, Tween 20) do not bind to the membrane, but flow through the column. Salts are quantitatively washed away by the ethanol-containing Wash III Solution. Any residual Wash III Solution, which may interfere with subsequent enzymatic reactions, is removed by an additional centrifugation step.

Elution in low-salt solutions
Elution efficiency is strongly dependent on the salt concentration and pH of the Elution Buffer. Contrary to adsorption, elution is most efficient under basic conditions and low salt concentrations. DNA is eluted with 50 or 30 ul of the provided Elution Buffer (10 mM Tris·Cl, pH 8.5), or Water. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water to elute, make sure that the pH is within this range. In addition, DNA must be stored at -20^{o}C when eluted with water since DNA may degrade in the absence of a buffering agent. Elution with TE (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) is possible, but not recommended because EDTA may inhibit subsequent enzymatic reactions.

DNA yield and concentration
DNA yield depends on the following three factors; the volume of Elution Buffer, how the buffer is applied to the column, and the incubation time of the buffer on the column. 100 ~ 200 ul of elution buffer completely covers the PCR Purification membrane, ensuring maximum yield, even when not applied directly to the center of the membrane. Elution with 50 ul requires the buffer to be added directly to the center of the membrane, and if elution is done with the minimum recommended volume of 30 ul, an additional 1 min incubation is required for optimal yield. DNA will be up to 1.7 times more concentrated if the PCR Purification column is incubated for 1 min with 30 ul of elution buffer, than if it is eluted in 50 ul without incubation.

Important Notes
Before equipment
- Add 100% ethanol to Wash III Solution before use (see bottle label for volume)
- All centrifuge steps are at 13,000 rpm (~ 17,900 X g) in a conventional tabletop microcentrifuge.

Vacuum notes
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- Wear safety glasses when working near a manifold under pressure
- The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere

Protocols
-Using a microcentrifuge
This protocol is designed to purify single- or double-stranded DNA fragments from PCR and other enzymatic reactions. For cleanup of other enzymatic reactions. Fragments ranging from 100 bps to 10 kbs are purified from primers, nucleotides, polymerases, and salts using spin columns in a microcentrifuge.
1. Add 5 volumes of PCR Purification Buffer to 1 volume of the PCR sample and mix.
   For example, add 500 ul of PCR Purification Buffer to 100 ul PCR sample (not including oil)
2. Place a spin column in a provided 2 ml collection tube
3. To bind DNA, apply the sample to the column and centrifuge for 30 ~ 60 sec.
4. Discard flow-through and place the column back in the same tube
   Collection tubes are reused to reduce plastic waste.
5. To wash, add 750 ul Wash III Solution to the column and centrifuge for 30 ~ 60 sec
6. Discard flow-through and place the column back in the same tube. Centrifuge the column for an additional 1 min.
   IMPORTANT : Residual ethanol from Wash III Solution will not be completely removed unless the flow-through is discarded before this additional centrifugation.
7. Place column in a clean 1.5 ml microcentrifuge tube
8. To elute DNA, add 50 ul Elution Buffer or Water to the center of the column membrane and centrifuge the column for 1 min. Alternatively, for increased DNA concentration, add 30 ul Elution Buffer to the center of each membrane, let the columns stand for 1 min, and then centrifuge.

-Using a vacuum manifold
1. Add 5 volumes of PCR Purification Buffer to 1 volume of the PCR sample and mix

For example, add 500 ul of PCR Purification Buffer to 100 ul PCR sample (not including oil)
2. Prepare the vacuum manifold and PCR Purification columns. Insert each spin column into a luer connector on the Luer Adapter in the manifold.
3. To bind DNA, load the samples into the spin columns by decanting or pipetting, and apply vacuum. After the samples have passed through the column, switch off the vacuum source. The maximum loading volume of the column is 800 ul. For sample volumes greater than 800 ul simply load again.
4. To wash, add 750 ul of Wash III Solution to each spin column and apply vacuum
5. Transfer each spin column to a microcentrifuge tube or the provided 2 ml collection tubes. Centrifuge for 1 min at 13,000 rpm (~ 17,900 X g).
   IMPORTANT : This spin is necessary to remove residual ethanol (Wash III Soluton)
6. Place each spin column into a clean 1.5 ml microcentrifuge tube.
7. To elute DNA, add 50 ul of Elution Buffer or Water to the center of each membrane, and centrifuge the columns for 1

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min at 13,000 rpm. Alternatively, for increased DNA concentration, add 30 ul Elution Buffer to the center of each membrane, let the columns stand for 1 min, and then centrifuge.

IMPORTANT : Ensure that the elution buffer is dispensed directly onto the membrane for complete elution of bound DNA.

Troubleshooting Guide

	Comments and suggestions
Low or no yield	
a) Wash III Solution did not contain ethanol	Ethanol must be added to Wash III Solution before use. Repeat procedure with correctly prepared Wash III Solution
b) Inappropriate elution buffer	DNA will only be eluted efficiently in the presence of low-salt buffer or water
c) Elution Buffer Incorrectly dispensed	Add Elution Buffer to the center of the membrane to ensure that the buffer completely covers the membrane. This is particularly important when using small elution volumes
DNA does not perform well the following step (e.g., Ligation)	
a) Salt concentration in elute too high	Modify the wash step by incubating the column for 5 mins at room temperature after adding 750 ul of Wash III Solution, the centrifuge.
b) Elution contains residual ethanol	Ensure that the wash flow-through is drained from the collection tube and that the spin column is then centrifuged at 13,000 rpm for an additional 1 min.
c) Elution contains primer-dimers	Primer-dirmers formed are longer than 20 bps, and are not completely removed. After the binding step, wash the spin column with 750 ul of 35% guanidine hydrochloride aqueous solution. Continue with the Wash III Solution wash step and the elution step as in the protocol.
d) Elute contains denatured ssDNA, which appears as smaller smeared band on an analytical gel	Use the eluted DNA to prepare the subsequent enzymatic reaction but omit the enzyme. To reanneal the ssDNA, incubate the reaction mixture at 95^{o}C for 2 min, and allow the tube to cool slowly to room temperature. Add the enzyme and proceed as usual. Alternatively, the DNA can be eluted in 10 mM Tris buffer containing 10 mM NaCl. The salt and buffering agent promote the renaturation of DNA strands. However, the salt concentration of the elute must be considered for subsequent applications.

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