Description:

The **Plus DNA Clean/Extraction Kit** is designed to extract DNA fragments of 70 bp to 50 kb from standard or low-melting agarose gels in either Tris acetate (TAE) or Tris borate (TBE) buffer system, and can also purify DNA fragments directly from an amplification or enzymatic reaction based on our specific buffer system. This kit can be used for DNA gel elution, PCR clean-up, and DNA desalting.

Features:

- Three different applications (PCR clean-up, DNA clean-up, gel extraction) with the same solutions.
- Up to 150 µl DNA solution or PCR reaction can be processed through each single Spin Column, from which 80%~95% of applied DNA can be recovered.
- DNA resolved on agarose gels, in both TAE and TBE buffers, can be purified. In a single Spin Column, up to 250 mg gel slice can be processed and 60~90% DNA recovery is expected.
- Purification process can be completed within 15 min.
- Phenol extraction or alcohol precipitation is not needed.
- Purified DNA is free from salt and macromolecular contaminants.

Components of the Kit:

	DP034P	**DP034P-300**
1. Binding Solution	60 ml	360 ml
2. Wash Solution	16 ml **(add 64 ml of Ethanol before use)**	96 ml **(add 384 ml of Ethanol before use)**
3. Elute Solution	10 ml	60 ml
4. Spin Column	50 pcs	150 pcs x 2
5. Collection Tube	50 pcs	150 pcs x 2

Materials to be supplied by the user:

- 100% Ethanol: For preparing the Wash Solution
- Water bath or heating block at 60°C

General Procedure for PCR and DNA Clean-Up:

1. Following PCR amplification or other enzymatic manipulations, transfer the *reaction mixture* (containing the DNA to be purified) into a clean microcentrifuge tube.
2. Add **3 volumes** of **Binding Solution** to the reaction mixture (e.g. 50 µl reaction mixture, add 150 µl Binding Solution) and vortex briefly to mix.
3. Insert the **Spin Column** into a **Collection Tube**, transfer the solution into the **Spin Column** and centrifuge at top speed (14~16,000 x *g*) for 1 min, and discard the filtrate in the collection tube.
   **If the sample volume exceeds 700 µl, load the remaining solution into the spin column and repeat this step.*
4. Add 700 µl of **Wash Solution** to the **Spin Column**, and wait for 1 min for equilibration with the membrane. Centrifuge at 14~16,000 x *g* for 1 min and discard the filtrate. Repeat this step once more.
5. Discard the filtrate and centrifuge at top speed for additional 5 min to remove residual trace of ethanol.
   ** If centrifugation speed is lower than 14,000 xg or residual ethanol must be removed completely, incubate the spin column in a heat oven (60~65°C) for 5 min to evaporate any residual ethanol.*
6. Transfer the **Spin Column** into a new microcentrifuge tube and add 30~100 µl of **Elute Solution (pH 7.0~8.5)** to the column and wait for 1~2 min.
7. Centrifuge at top speed for 2 min to elute the DNA. Store the eluted DNA at -20°C.

General Procedure for gel extraction:

1. Following electrophoresis, cut out the desired DNA band (≤ 250 mg) from agarose gel in TAE or TBE buffer.
   **Up to 250 mg gel slice can be purified in one column purification.*
2. Transfer the gel slice into a 1.5 ml clean microcentrifuge tube and add **2 volumes** (or 3 volumes if agarose gel > 2%) of **Binding Solution** to the gel slice, and incubate at 60°C for 5~15 min or longer until gel slice has completely dissolved. Vortex the tube to mix every 2~3 min during incubation.
3. Insert the **Spin Column** into a **Collection Tube**, transfer the solution into the **Spin Column** and centrifuge at top speed (14~16,000 x *g*) for 1 min. Discard the filtrate in the collection tube.
   **If the sample volume exceeds 700 µl, load the remaining solution into the spin column and repeat this step.*
4. **Optional:** Add 500 µl of **Binding Solution** and centrifuge at top speed for 1

min. Discard the filtrate in the collection tube.

***This step will remove residual agarose which may inhibit enzymatic reaction of purified DNA.*

5. Add 700 µl of **Wash Solution** to the **Spin Column**, and wait for 1 min for equilibration with the membrane. Centrifuge at 14~16,000 x *g* for 1 min and discard the filtrate. Repeat this step once more.
6. Discard the filtrate and centrifuge at top speed for additional 5 min to remove residual trace of ethanol.
   If centrifugation speed is lower than 14,000 xg or residual ethanol must be removed completely, incubate the spin column in a heat oven (60~65℃) for 5 min to evaporate any residual ethanol.
7. Transfer the **Spin Column** into a new microcentrifuge tube and add 30~100 µl of **Elute Solution (pH 7.0~8.5)** into the column and wait for 1~2 min.
8. Centrifuge at top speed for 2 min to elute the DNA. Store the eluted DNA at -20°C.

Troubleshooting Guide

Problem	Comments and Suggestion
Low DNA recovery	
a) Wash Solution was not diluted properly Insufficient / no PCR product	Make sure to add ethanol to Wash Solution before use. Estimate DNA recovery by running 10% of PCR product before and after purification on an agarose gel.
b) Gel slice dissolved incompletely	1) If using more than 250 mg of gel slice, separate it into multiple tubes. 2) Cut large gel slices into several pieces to accelerate the gel dissolution.
c) DNA was not eluted properly.	1) Extend the incubation time to completely dissolve the gel slice. 2) For DNA are larger than 5 Kb, use preheated Elute Solution (60~70 °C). 3) DNA is eluted only in the presence of low-salt Solution (e.g., Elute Solution [10 mM Tris·Cl, pH 8.5]). Elution efficiency is dependent on pH. The maximum efficiency is achieved

	between pH 7.0 and 8.5. 4) Ensure that Elution Solution is added into the center of the membrane and is completely absorbed. 5) Allow the Elute Solution to incubate in the column for longer time.
Poor performance in downstream applications DNA eluate is contaminated with salt. a) Residual ethanol contamination b) DNA was denatured	 Do not allow the binding or wash flow-through liquid to come in contact with the bottom of the binding column following the spin steps. Wash the column twice with Wash Solution. Be sure to centrifuge at 14~16,000 x *g* for 5 minutes in the Step 6 and incubate the column in oven at 60~65 ℃ oven for 5 minutes to evaporate of the ethanol. Add 1/10-1/5 volume of Elute Solution to the eluate, incubate at room temperature for 5 min. Alternatively, incubate the DNA Solution at 95 ℃ for 2 min, then cool down slowly to re-anneal the denatured DNA.
Low A_{260}/A_{230} Guanidine isothiocyanate contamination	1) In the wash step, repeat the 700 µl of Wash Solution addition and let stand for 1 minute. 2) Ethanol precipitate the DNA if low A_{260}/A_{230} ratio is a concern.