Cyclo-Prep™, 2 in 1 DNA Isolation Kit (K600-50RXN)

* Fast spin column format to isolate both PCR products and DNA from agarose gels
* Purified DNA is free of any salt or macromolecular contaminants
* High yields without using phenol or alcohol precipitation

Product Description:

Cyclo-Prep, 2 in 1 Kit is a spin column-based kit for the quick isolation of both PCR products (> 100bp) and DNA fragments from agarose gels. In just 10 minutes, isolate ultra-pure DNA products ready-to-use in restriction enzyme digestion, labeling, hybridization, ligation, TA cloning, and fluorescent sequencing protocols.

Kit Components:

The Cyclo-Prep, 2 in 1 Kit contains reagents sufficient for 50 purifications. All reagents can be stored at room temperature. Each kit includes the following components.

* **Binding Buffer** 55 mL
* **Washing Buffer** 16 mL (Add 64 mL of Ethanol before use)
* **Elution Buffer** 10 mL
* **Spin Columns** 50 each
* **Collection Tubes** 50 each

Required Equipment: A micropipettor, bench-top microcentrifuge, and 1.5 - 1.7 ml microfuge tubes.

Protocol for PCR and DNA Clean-up:

1. Transfer the aqueous layer of the PCR amplification or other DNA solution (after enzymatic treatment) to a clean sterile micro-centrifuge tube.
2. For DNA solutions ≤ 100 uL, add 500 uL of **Binding Buffer** and vortex briefly.
   Note: For DNA solution volumes > 100 uL, add 5 volumes of **Binding Buffer**. For sample volumes exceeding the capacity of the spin column (750 uL), repeat the column loading/spin in steps 3 and 4 for additional sample
3. Insert a **Spin Column** into a **Collection Tube**. Transfer the solution to the Spin Column and spin at top speed (12-14,000 x g) for 1 min.
4. Remove the Spin Column from the Collection Tube, discard the filtrate.
5. Add 700 ul of the **Washing Solution** to the spin column and spin at (12-14,000 x g) for 1 min. **Note:** For fluorescent sequencing, a repeat wash is recommended.
6. Remove the spin column from the collection tube and discard the filtrate. Spin for an additional 3 min (12-14,000 x g) to remove residual traces of ethanol. **Note:** Spin column can be placed in a 37 – 60°C oven for 5 min (no longer than 10 min) to evaporate all ethanol prior to eluting the DNA (For DNA used in fluorescent sequencing, this incubation is recommended).

7. Remove the spin column and place it into a new micro-centrifuge tube (not provided). Add 30-50 ul of **Elution Buffer** or $H_2O^{a,b}$. Spin at 12-14,000 x g for 1 min. **Note:** For DNA fragments > 5 kb, use preheated (60-70°C) H_2O or TE Buffer to elute.
8. Store the eluted DNA at –20°C.

a. For fluorescent sequencing, use only H_2O to elute DNA.
b. Water with a low pH (< 7.0) may cause lower DNA recovery.

Protocol for DNA Purification from Agarose Gels:

1. Excise the desired DNA band (≤ 350 mg) from the agarose and place it into a clean sterile micro-centrifuge tube.
2. Add an equal volume of **Binding Buffer** to the gel slice and incubate at 60°C for 5-15 minutes (or until completely dissolved). **Note:** For > 2% gel, add 2-3 volumes of Binding Buffer.
3. Insert a **Spin Column** into a **Collection Tube**. When the gel slice is completely melted, apply the melted solution to the Spin Column and spin at 12-14,000 x g for 1 min. Remove the Spin Column from the Collection Tube, discard the filtrate.
4. *Optional Step:* Add 500 uL of **Binding Buffer** and spin at 12-14,000 x g for 1 min. Remove the Spin Column from the Collection Tube, discard the filtrate. **Note:** This step will remove any residual agarose which might inhibit enzymatic reactions of the purified DNA. (Recommended for agarose >2% and DNA for fluorescent sequencing).
5. Add 700 ul of the **Washing Solution** to the spin column and spin at (12-14,000 x g) for 1 min. **Note:** For fluorescent sequencing, a repeat wash is recommended.
6. Remove the spin column from the collection tube and discard the filtrate. Spin for an additional 3 min (12-14,000 x g) to remove residual traces of ethanol. **Note:** Spin column can be placed in a 37 – 60°C oven for 5 min (no longer than 10 min) to evaporate all ethanol prior to eluting the DNA (For DNA used in fluorescent sequencing, this incubation is recommended)
9. Remove the spin column and place it into a new micro-centrifuge tube (not provided). Add 30-50 ul of **Elution Buffer** or $H_2O^{a,b}$. Spin at 12-14,000 x g for 1 min. **Note:** For DNA fragments > 5 kb, use preheated (60-70°C) H_2O or TE Buffer to elute.
10. Store the eluted DNA at –20°C.

a. For fluorescent sequencing, use only H_2O to elute DNA.
b. Water with a low pH (< 7.0) may cause lower DNA recovery.

The Polymerase Chain Reaction (PCR) is covered by patents owned by Hoffman-La Roche

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