Note: *for laboratory research use only.*

Gel Extraction & PCR Purification Combo Kit (Spin-column)

Cat#： DP1502 (100 preps)

Signalway Biotechnology

I. Kit Content Storage and Stability

Contents	Storage	100T (DP1502)
Buffer DB	RT	100 ml
Buffer WB	RT	25 ml
		Add 100ml ethanol before use.
Buffer EB	RT	20 ml
Sodium Acetate (3 M, pH 5.2)	RT	0.5 ml
Spin-column AC	RT	100
Collection Tube (2 ml）	RT	100

All reagents are stable for up to 12 months if stored properly.

Reminder:

- ***Please add 100 ml ethanol to buffer WB before use; mix adequately and then check the box on the label showing ethanol was added!***
- Buffer may precipitate under low temperature. Incubate the buffer at 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.
- Keep all of the reagents lids tightly caped when not in use to prevent evaporation, oxidation and changes in pH.

II. Principle

DNA fragments bind to silica membrane in high salt buffer. Utilize a series of elution-centrifugation steps to remove cellular metabolites and proteins. DNA fragments are eluted in low salt and high pH buffer.

III. Features

1. Rapid and convenient. No poisonous phenol contained. No need for ethanol precipitation.
2. High-quality binding solution will not inhibit down-stream reactions because no sodium iodide or perchlorate is used.
3. Binding buffer DB is yellow, which is convenient for monitoring the pH when melting gel.
4. The recovery efficiency for short fragments can reach 95%.

IV. Notes

1. All the centrifugation can be performed at room temperature.
2. The size of DNA fragments for gel purification ranges 100 bp-50 kb.
3. The yields of DNA depend on initial amount of DNA, elution volume, and the size of the DNA fragments. Usually, the recovery efficiency is 85%-95% when DNA is 5-25 μg and 100 bp-5 kb long.
4. Because of UV damage, please use long wave UV light and shorten operation time.
5. Monitor the pH of the gel/buffer mixture after the gel has completely dissolved. If the color of the mixture becomes orange or heliotrope, add 5 μl -10 μl of 3 M sodium acetate (pH 5.2) to justify the pH down to 5-7. After this adjustment, the color of the gel/buffer mixture should be light yellow.
6. There is no EDTA in Buffer EB, which should not affect down-stream reactions. If interfere occurs, use sterile water to elute DNA, but ensure pH >7.5, and then store at -20°C. Low pH will decrease the elution efficiency. For long-term storage, dissolve plasmid in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Be sure to dilute the DNA solution before use because the EDTA will affect down-stream reactions.

V. Procedure

Dilute Buffer WB with 100 ml absolute ethanol before starting.

● For Gel extraction

1. Carefully excise the DNA fragment of interest using a clear, sharp scalpel under the UV light. Minimize the size of gel slice by removing extra agarose.
2. Determine the approximate volume of the gel slice by weighting it in a 1.5 ml tube that has been weighted before using.
3. Add three volume of Buffer DB.

   A 100 mg gel slice is about 100 µl and add 300 µl Buffer DB; If the concentration of gel is ≥2%, please add six volume of Buffer DB;

   Avoid using gel more than 400mg! If do, use multiple spin-columns.
4. Incubate the mixture at 56°C water-bath for 3-5 minutes until the gel has completely melted. Vortex the tube every 1-2 minutes.
5. Place Spin-column AC into Collection Tube and load the mixture of step 4 into Spin-column AC. Centrifuge at 13,000 rpm for 30-60 seconds. Discard the flow-through. (Repeat the step until all mixture is loaded.)
6. Add 700 µl buffer WB (**Please check if ethanol is added!**) Centrifuge at 13,000 rpm for 1 minute. Discard the flow-through.
7. Centrifuge the Spin-column AC at 13,000 rpm for 2 minutes.
8. Transfer the Spin-column AC to a clean microcentrifuge tube. Add 50 µl buffer EB. (pre-warmed at 65-70°C) and incubate for 2 minutes at room temperature.
   Centrifuge at 13,000 rpm for 1 minute.
9. For maximum efficiency, repeat step 8

   The volume of elution buffer could be adjusted. Appropriately reduce elution volume can increase concentration. However, the minimum volume is 30 µl. If the elution volume is less than 30 µl, the yield of DNA will be decreased.
10. Keep DNA at -20°C or apply to down-stream reactions

Dilute Buffer WB with 100ml absolute ethanol before starting.

● For PCR or digestion products

1. The initial volume is 100 µl. Add 500 µl buffer DB into the PCR or digestion reaction system.

   If the initial volume of system is less than 100 µl, add sterile water to make the final volume 100 µl

2. Place Spin-column AC into Collection Tube and load the mixture of Step 1 into Spin-column AC. Incubate for 5 minutes at room temperature. Centrifuge at 13,000 rpm for 30-60 seconds, and discard the flow-through.
3. Add 700 µl buffer WB (**check if ethanol has been added!**). Centrifuge at 13,000 rpm for 1 minute. Discard the flow-through.
4. Centrifuge the Spin-column AC at 13,000 rpm for 2 minutes.
5. Transfer the Spin-column AC to a clean microcentrifuge tube. Add 50 µl buffer EB (pre-warmed at 65-70°C) and incubate for 2 minutes at room temperature. Centrifuge at 13,000 rpm for 1 minute.
6. For maximum efficiency repeat step 5

   The volume of elution buffer could be adjusted. Appropriately reduce elution volume can increase concentration. However, the minimum volume is 30 µl. If the elution volume is less than 30 µl, the yield of DNA will be decreased.
7. Keep DNA at -20°C or apply to down-stream reactions

Ⅵ. Troubleshooting

Problems	Causes	Solutions
Low yield of purified DNA	Inappropriate storage of the kit.	Store the kit at RT (15℃－20℃)
	The buffers are under environments that affect quality.	Store at RT (15℃－20℃) Ensure all of buffer capped tightly when not in use, preventing reagents evaporating, oxidation, and pH change.
	Forget to add ethanol to Buffer WB.	Add 100 ml ethanol before use.
	Not thoroughly mixed when add Buffer DB into products	Mix thoroughly
Low efficient elution	High pH Buffer EB is very important.	Use the Buffer EB in the kit, not water.
A_{260} too high	The eluted silica matrix affects A_{260} value.	Centrifuge at 13,000 rpm for 1 minute to spin down debris. Use the supernatant
No DNA in flow-through	There is no DNA in initial sample.	Please ensure before extracting.
DNA concentration is too low	It is too low in the initial sample.	Increase the volume of initial sample and decrease that of Buffer DB, but not < 30 μl.
	The DNA fragment is <100bp, or >10kb.	Increase the amount of initial sample.
Low recovering yield	Not enough Buffer DB for gel extraction.	Ensure the ratio of gel/Buffer DB is 1:3. The Liquid Paraffin and loading buffer will not affect extracting.
	Not fully eluted	Elute twice (once with 30 μl elution buffer).