

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

DP1501 50 preps

Content Storage

Contents	Storage	Volume
Buffer DB	RT	50 ml
Buffer WB	RT	15 ml
	Add the ration ethanol before use	
Buffer EB	RT	15 ml
Isopropanol	RT	15 ml
Sodium Acetate 3 M (pH 5,2)	RT	0,5 ml
Spin-Column AC	RT	50
Collection Tube (2 ml)	RT	50

All reagents are stable for 12 months at RT.

15 ml Buffer WB must be diluted with 60 ml absolute ethanol before starting.

Precipitates may form in buffers when stored at low temperature. Warm at 37°C to dissolve.

Storage and transportation at room temperature 15°C -25°C. Ensure the bottles of buffer are tightly capped when not in use, preventing reagents evaporating, oxidation and pH change.

Principle

DNA fragments bind to silica membrane in high salt buffer. Cellular metabolite and proteins are removed by a serial of elution- centrifuge steps. Then DNA fragments are eluted in low salt and high pH buffer.

Features

1. Do not contain poisonous phenol and do not need ethanol precipitation.
2. Best quality binding solution, without sodium iodide and perchlorate, no inhibition effect to down-stream reactions.
3. The binding buffer DB is yellow, which is convenient for monitoring the pH when melting gel.

Notes

1. All the centrifugation steps can be performed at room temperature.
2. Buffer P3 contains stimulating compound. Please wear latex gloves, avoiding skin, eyes and cloth to be contaminated. **If that, please use water or physiological saline washing.**
3. The best size of DNA fragments for gel purification is between 100bp-50kb. Too short fragments may decrease the yield.
4. The yields of DNA have relation with initial DNA concentration, elution volume and the size of the DNA fragment. Usually, the efficiency is 85% - 95%, when DNA is 5-25 µg and 100bp-5kb.
5. Because of UV damage, please use length wave UV ray and shorten operation time.
6. Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. If the color of the mixture becomes orange or heliotrope, add 5µl -10µl of 3M sodium acetate, pH 5.2 to bring the pH down to 5-7. **After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.**
7. There is no EDTA in Buffer EB, which cannot influence down-stream reactions. Also you can use water when eluting, but please ensure pH>7.5 and store at -20°C. If for long-term storage, dissolve plasmid DNA in TE (10mM Tris-HCl, 1mM EDTA, pH 8.0). Because EDTA will affect the down-stream reactions, dilute the solution before use.

Procedure for agarose gel DNA purification

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 was weighted before using.
3. Add three volume of Buffer DB. A gel slice of mass 0.1g will have a volume of 100 µl and add 300 µl Buffer DB; If the concentration of gel is ≥2%, please add six volume of Buffer DB. Do not let the mass of gel exceed 400 mg! If do, please use multiple Spin-column to reclaim.

4. Incubate the mixture at 56°C water-bath for 3-5 min or until the gel has completely melted. Mix by vortexing the tube every 1-2 min. Add 150 µl of Isopropanol per 100 mg of gel slice and mix well.
Isopropanol can increase the yield of DNA only if the fragment is less than 4 kb. If the band is over 4 kb, do not add the Isopropanol.
5. Place Spin-column AC into Collection Tube and add the mixture of step 4 into Spin-column AC. Centrifuge at 12,000rpm for 30-60 sec. Discard the flow-through.
6. Add 700 µl buffer WB (**please check if ethanol added!**). Centrifuge at 12,000 rpm for 1 min. Discard the flowthrough.
7. Centrifuge the empty Spin-column AC at 12,000 rpm for 2 min to dry the column.
8. Transfer the Spin-column AC to a clean tube. Add 50 µl buffer EB (having been incubated at 65-70°C water-bath) and incubate for 2 min at room temperature. Centrifuge at 12,000 rpm for 1 min. An optional, second elution will yield any residual DNA. The volume of elution buffer could be adjusted. Appropriately reduce elution volume can increase concentration, but the minimum volume is 30 µl. If the elution volume is less than 30 µl, the final yield of DNA will be affected.

Procedure for PCR or digestion purification

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

If the volume of system is less than 100 µl, please add sterile water up to 100 µl.

2. Place Spin column AC into collection tube and add the mixture of step 1 into spin column AC. Incubate for 5 min at room temperature. Centrifuge at 12,000 rpm for 30-60 s. Discard the flow-through.
3. Add 700 µl buffer WB (**check if ethanol is added!**). Centrifuge at 12,000 rpm for 1 min. Discard flow-through.
5. Centrifuge empty Spin column at 12,000 rpm for 2 min to dry the column.
6. Transfer the spin column to a clean tube. Add 50 µl buffer EB (pre-warmed to 65°C -70°C before using) directly onto the column and incubate for 2 min at room temperature. Centrifuge at 12,000 rpm for 1 min. An optional second elution will yield any residual DNA.

The volume of elution buffer can be adjusted according to needs. Appropriately reduce elution volume can increase concentration, but the minimum volume is 30 µl, too low elution volume will decrease the elution efficiency and the final DNA yield.

Troubleshooting

Problems	Causes	Advices
Low yield or not purity	Non-optimal temperature for the kit	Store the kit at RT (15°C -20°C)
	The buffers are under environments that influence their quality	Store at RT (15°C -20°C). Ensure all bottles of buffer capped tightly when no in use
	Forget adding ethanol to Buffer WB	Add the ration ethanol before use
	Not thoroughly mixed when adding Buffer DB into products	Should mix thoroughly
Low efficiency elution	High pH Buffer EB is very important	Using the Buffer EB in the Kit, not water.
A260 too high	The eluted Silica matrix affects A260 value	Centrifuge at 13,000 rpm for 1 minute, carefully using the supernatant
DNA concentration It is too low	It is too low DNA concentration in the initial sample	Increase the volume of initial sample and decrease that of Buffer DB, but not under 30ul
	The DNA fragment is <100bp, or >10kb	Increase the volume of initial sample