

## **Gel DNA Purification Kit (Spin-column)**

**Cat. # DP1701 (50 preps)**

**DP1702 (100 preps)**

**DP1703 (200 preps)**

---

**One Innovation Drive, Worcester, MA 02446, USA**

**Phone: (508)-421-4800; 877-421-4800 (Toll Free);**

**Fax: 508-421-4801**

**Emails: [info@biocytogen.com](mailto:info@biocytogen.com); [order@biocytogen.com](mailto:order@biocytogen.com)**

## I. Kit Content Storage and Stability

Contents	Storage	50 preps (DP1701)	100 preps (DP1702)	200 preps (DP1703)
Buffer DB	RT	20 ml	40ml	80 ml
Buffer WB	RT	15 ml	15ml	25 ml
		<i>Add the ration ethanol before use.</i>		
Buffer EB	RT	15 ml	20 ml	20 ml
3M Sodium Acetate (pH5.2)	RT	0.5ml	0.5ml	0.5 ml
Spin-column AC	RT	50	100	200
Collection Tube (2ml)	RT	50	100	200

**All reagents are stable for 12 months, when stored properly.**

### Notes:

- Buffer WB must be diluted with four volumes absolute ethanol before starting.
- Precipitate may form in buffers when stored at low temperature. Warm at 37°C to dissolve it.
- Precipitates may form in buffers, which will affect the efficiency when stored at 4°C or -20°C. Storage and transportation at room temperature (15°C -25°C).
- Ensure the bottles of buffer tightly capped when not in use, preventing reagents evaporating, oxidation and pH change.

## II. Principle

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

### III. Features

- Do not contain poisonous phenol and do not need ethanol precipitation.
- Best quality binding solution, without sodium iodide and perchlorate, has no inhibition to downstream reactions.
- The binding buffer DB is yellow, which is convenient for monitoring the pH when melting gel.
- The silica membranes in the spin-column come from the world-famous company. The yield for short fragments can achieve 75%.

### IV. Notes

1. All centrifugation steps can be performed at room temperature.
2. Buffer P3 contains the stimulating compound. Please wear latex gloves, avoiding skin, eyes and cloth to be contaminated. **If that, please wash with water or physiological saline.**
3. The size of DNA fragments between 100bp-50kb for gel purification is better. Too short may decrease the yield.
4. The yields of DNA have relations with initial DNA concentration, elution volume and the size of the DNA fragment. Usually, the efficiency is 85%-95%, when DNA is 5-25 $\mu$ g and 100bp-5kb.
5. Because of UV damage, please using 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 5 $\mu$ l -10 $\mu$ l 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. No EDTA in Buffer EB, which will have no influence on down-stream reactions. Also you can use water when eluting, but please ensure PH>7.5 and store at -20 $\square$ . If for long-term storage, dissolve plasmid in TE(10mM Tris-HCl, 1mM EDTA, PH 8.0). Because EDTA will affect the down-stream reactions, dilute the solution before using.

## V. Procedure

*Note: Diluted buffer WB with four volumes absolute ethanol before starting*

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. Determined the approximate volume of the gel slice by weighting it in a 1.5ml tube that has been weighted before use.
3. Add three volume of Buffer DB.

**A gel slice of mass around 0.1g will have a volume of 100 $\mu$ l and add 300 $\mu$ l Buffer DB;**

**If the concentration of gel is  $\geq 2\%$ , please add six volumes of Buffer DB;**

**The mass of gel should be less than 400mg! If necessary, please use multiple spin-columns to reclaim.**

4. Incubate the mixture at 56 $^{\circ}$ C for 3-5 min or until the gel has completely melted. Mix by vortexing the tube every 1-2 min.
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 $\mu$ l buffer WB (**please check ethanol added!**). Centrifuge at 12,000 rpm for 1min. Discard the flow-through.
7. Centrifuge the empty spin-column AC at 12,000 rpm for 2min to dry the column.
8. Transfer the spin-column AC to a clean tube. Add 50 $\mu$ l buffer EB (having been incubation 65-70 $^{\circ}$ C), 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 according to needs.**

**Appropriately reduce elution volume can increase concentration. But the minimum volume is 30 $\mu$ l, too less will decrease the elution efficiency and the DNA yield.**

## VI. Troubleshooting

Problems	Causes	Advices
Low yield or not purity	Non-optimal for the kit	Store the kit at RT (15°C-20°C)
	The buffers is under environment that influence the effect.	Store at RT (15°C-20°C) Ensure all bottles of buffer capped tightly when no in use, preventing reagents evaporating, oxidation and PH change.
	Forget adding ethanol to Buffer WB.	<b>Add the ration ethanol before use.</b>
	Not thoroughly mixed when adding Buffer DB into products.	<b>Mix thoroughly.</b>
Low efficient elution	High pH Buffer EB is very important.	Using the Buffer EB in the Kit, not water.
A <sub>260</sub> too high	The eluted Silica matrix influence A <sub>260</sub> value.	Centrifuge at 13,000 rpm for 1 minute, carefully using the supernatant.
No DNA in flow-through	There is no DNA in initial sample.	Please check samples 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 <30µl.
	The DNA fragment is <100bp, or >10kb	Increase the volume of initial sample.
Low extracting yield	Not enough Buffer DB for gel extraction.	Ensure the ratio of gel/Buffer DB is 1:5. And the Liquid Paraffin and loading buffer will not affect extracting.
	Not fully eluted	Twice elution (once is 30µl )