

## **Plasmid DNA Maxi-Preparation Kit (Spin-column)**

Cat. # DP2501 (5 preps)  
DP2502 (10 preps)

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## I. Kit Content, Storage and Stability

Content	Storage	5 preps ( DP2501 )	10 preps ( DP2502 )
RNase A	-20□	400μl(10mg/ml)	750μl(10mg/ml)
Buffer P1	4□	50 ml	100 ml
Buffer P2	RT	50 ml	100 ml
Buffer P3	RT	75 ml	150 ml
Buffer PE	RT	50 ml	100 ml
Buffer WB	RT	15 ml	50 ml
		<i>Add the ration ethanol before use.</i>	
Buffer EB	RT	15 ml	20 ml
Spin-column AC	RT	5	10
Collection Tube ( 50ml )	RT	5	10

All reagents are stable for 12 months when stored properly.

### Notes:

1. *Please add the ration ethanol into Buffer WB before use, vortex adequately, and then mark the bottle to avoid multi-adding!*
2. *Please add all the RNase A in the tube into Buffer P1 before starting; the final concentration is 100mg/ml, then store at 4□*
3. Buffer P2 may precipitate under low temperature. Incubate to 37for a moment **until clear**, then cool down to RT for use.
4. Please keep all reagents' lids tightly when not in use to prevent reagents evaporating, oxidation and pH change.

## II. Principle

The kit applies the improved SDS method to rapidly lyse cells, then DNA selectively adsorbs on silica membrane in high salt solution. Take a serial of elution-centrifugation steps to remove cellular metabolites and proteins etc. Finally use low salt elution to elute purified genome DNA from silica membrane.

### III. Features

1. Poisonous phenol etc is not used in this kit.
2. Multi-elution can ensure high-quality DNA, which are suitable for all kinds of molecular experiments such as PCR, Southern-blot and enzyme digestion directly.
3. The silica membranes in the spin-column come from the world-famous company.
4. The yield of plasmid is around 0.5-1mg/100-500ml culture.

### IV. Notes

1. **All the centrifugation steps can be performed at RT** and 8,000 rpm in traditional centrifuge with 50ml rotor.
2. Buffer P2 contains the stimulating compound; please wear latex gloves to avoid skin, eyes and cloth to be contaminated. **If that, please wash with water or physiological saline.**
3. **The yield of plasmid depends on culture concentration and plasmid copy number.** For stringent plasmid or the size >10kb, increase the volume of inoculated LB for extracting, with rational increasing volume of Buffer P1, P2 and P3.
4. The agarose gel electrophoresis and UV—spectrometer can be used for detecting the concentration and purity of the plasmid. 1OD<sub>260</sub> may be 50µg/ml DNA. The supercoiled plasmid conformations may display different types, single or two even more bands in agarose gel electrophoresis, because their types are influenced by culture time and operations of extracting.
5. Please digest plasmid to check the exact size when compared with DNA marker.
6. There is no EDTA in Buffer EB, which will not affect down-stream reactions. Also use water to elute DNA, but please ensure pH>7.5, and store at -20°C. Low pH will decrease the elution efficiency. For long-term storage, dissolve plasmid in TE (10mM Tris-HCl, 1mM EDTA, pH 8.0). But please dilute the DNA solution before use because EDTA will affect the down-stream reactions.

## V. Procedure

**Please add 75 ml ethanol to 25ml Buffer WB (5 preps, DP2501) or 150ml ethanol to 50ml Buffer WB (10 preps, DP2502)!**

**Please add all RNase A in the tube into Buffer P1 before starting; the final concentration is 100mg/ml, then store at 4°C**

**Please check the solution for precipitate, and incubate at 37°C until clear if necessary!**

**Please keep Buffer P2 lid tightly after use!**

1. Harvest culture (100-500ml) in a 50 ml centrifuge tube by centrifuging at 8,000rpm for 10-15min. Discard supernatant as far as possible.

2. Add 9ml Buffer P1 and suspend cells completely.

**If not, affect cell lysis and seriously decrease yield.**

3. Add 9ml Buffer P2, and then overturn 6-10 times to mix thoroughly and gently until clear (about 4min).

**Please overturn to mix thoroughly and complete this procedure in 5 minutes, avoiding breaking plasmid.**

4. Add 14.4ml Buffer P3, then **overturn to mix thoroughly and gently until** the flocculated precipitate appears, and incubate at RT. Then centrifuge at 8,000rpm for 15min.

5. Place Spin-column AC into Collection Tube. Add the supernatant from step 4 into Spin-column AC (once 10ml), centrifuge at 8,000rpm for 3min, discard flow-through. Repeat until all supernatant added.

6. Add 10ml Buffer PE, centrifuge at 8,000rpm for 5min, and discard flow-through.

7. Add 10ml Buffer WB (**please check if ethanol added!**), centrifuge at 8,000 rpm for 3min, and discard flow-through.

8. Repeat step8.

9. Place the empty Spin-column AC back on the Collection Tube, centrifuge at 8,000 rpm 5min.

10. Transfer the Spin-column AC to a clean tube, add 1.5ml buffer EB (having

been incubated at 65-70°C, stay for 1 min at RT, then centrifuge at 10,000 rpm for 3min. Buffer EB is Tris-HCl (pH 8.0), does not affect down-stream reaction.

## VI. Troubleshooting

Problem	Reason	Solutions
Low yield	No antibiotic in culture, which cause the non-transformants over-growth.	Ensure the liquid and solid culture contains the antibiotic.
	Time of culturing is too long; the old cells begin lyse.	Inoculate fresh cells into liquid culture and the time of culturing is not over 16 hours.
	Use stringent plasmid	Advice using the relaxed plasmid, or increasing volume of treatment.
	The concentration is too low or not enough.	Harvest cells until the $[A_{600}] = 2-4$ .
	Cells not lysed adequately.	Please don't treat too much cells; suspend cells completely in Buffer P1; After add Buffer P2, the mixture should be sticky and transparent.
	By UV—spectrometer, the concentration usually is on the high side.	Use the agarose gel electrophoresis to determine concentration.
	Low elution efficiency.	<b>Please read step10-12 and Notes 6 before starting.</b>
No product	Ethanol not added to Buffer WB.	<b>Add the ration ethanol before use.</b>
	There is too much ethanol in the elution buffer; and the DNA float out the lanes before electrophoresis.	Ensure have had step10, and no ethanol remains; Increase the volume of loading buffer.

DNA digestion inhibition	Eluted silica membrane inhibits digestion.	Centrifuge at 8,000 rpm for 1 minute, carefully take the supernatant to remove contaminant.
	Ethanol remains in spin-column or collection tube bottom.	Ensure do step 10, and wait for a moment to do next step.
Contaminated with genomic DNA	In the process of isolation, the genomic DNA is broken.	Do step 3 and <b>overturn to mix thoroughly and gently</b> . Do not vortex and shake rigorously.
Nicked plasmid or denatured plasmid band appeared in front of supercoiled one	Time for step 3 is too long.	<b>Please do step 3 in 5 min.</b>
Contaminated by RNA	RNaseA not added into Buffer P1; Too much cells treated; or RNaseA is inactive.	Ensure add RNase A into Buffer P1; If Buffer P1 have been stored over 3 months, then add new RNase A into it; Don't treat too much cells; when cells are suspended in Buffer P1, please wait a moment for RNase A reaction.