

Plasmid DNA MAXI-Preparation Kit

(Spin-column)

For laboratory research use only

DP2501 **5 preps**

Kit Content, Storage, and Stability

Content	Storage	5 preps
RNase A	-20°C	400 ul (10 mg/ml)
Buffer P1	4°C	50 ml
Buffer P2	RT	50 ml
Buffer P3	RT	75 ml
Buffer PE	RT	50 ml
Buffer WB	RT	25 ml
	Add ration ethanol before use	
Buffer EB	RT	15 ml
Spin-column AC	RT	5
Collection Tube (50 ml)	RT	5

All reagents are stable for 12 months when stored properly.

I. 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 100 mg/ml, then store at 4°C.
3. Buffer P2 may precipitate under low temperature. Incubate to 37°C for 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 absorbs 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 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 50 ml 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. 1OD260 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 25 ml Buffer WB (5 preps, DP2501).
 - ✓ Please add all RNase A from the tube into Buffer P1 before starting; the final concentration is 100mg/ml, then store at 4°C.
 - ✓ Please check the solution. If precipitated, incubate at 37°C until clear if necessary!
 - ✓ Please keep Buffer P2 lid tightly after use!
1. Harvest culture (100-500 ml) in a 50 ml centrifuge tube by centrifuging at 8,000 rpm for 10-15 min. Discard supernatant as much as possible.
 2. Add 9 ml Buffer P1 and suspend cells completely. If not, affect cell lysis and seriously decrease yield.
 3. Add 9 ml Buffer P2 and then overturn 6-10 times to mix thoroughly and gently until clear (about 4 min). Please overturn to mix thoroughly and **complete this procedure in 5 minutes**, avoiding breaking plasmid.
 4. Add 14.4 ml Buffer P3, then overturn to mix thoroughly and gently until the flocculated precipitate appears and incubate at RT. Then centrifuge at 8,000 rpm for 15 min.
 5. Place Spin-column AC into Collection Tube. Add the supernatant from step 4 into Spin-column AC (once 10 ml), centrifuge at 8,000 rpm for 3 min, discard flow-through. Repeat until all supernatant added.
 6. Add 10 ml Buffer PE, centrifuge at 8,000 rpm for 5 min and discard flow-through.
 7. Add 10 ml Buffer WB (please check if ethanol added!), centrifuge at 8,000 rpm for 3 min, and discard flow-through.
 8. Repeat step 7.
 9. Place the empty Spin-column AC back on the Collection Tube, centrifuge at 8,000 rpm 5 min.
 10. Transfer the Spin-column AC to a clean tube, add 1.5ml buffer EB (incubated at 65-70°C), stay for 1 min at RT, then centrifuge at 10,000 rpm for 3 min. Buffer EB is Tris-HCl (pH 8.0), it does not affect down-stream reaction.

VI. Troubleshooting

Problem	Reason	Solutions
Low yield	No antibiotic in culture, which cause the nontransformants 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.	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 increase volume of treatment.
	The concentration is too low or not enough.	Harvest cells until the [A600] =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	Please read step10-12 and Notes 6 before starting.
No product	Ethanol not added to Buffer WB	Add the ration ethanol before use.
	There is too much ethanol in the elution buffer, 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 overturn to mix thoroughly and gently. Do not vortex and shake rigorously
Nicked plasmid or denatured plasmid band appear in front of supercoiled one	Time for step 3 is too long.	Please do step 3 in 5 min
Contamination by RNA	RNase A not added into Buffer P1; too much cells treated; or RNaseA is inactive.	Ensure add RNase A into Buffer P1; If Buffer P1has been stored over 3 months, then add new RNase A into it. Do not treat too much cells; when cells are resuspended in Buffer P1, please wait a moment for RNase A reaction.