Note: *for laboratory research use only*

Plasmid DNA Maxi-Preparation Kit

(Spin-column)

Cat. # DP2502 (10 preps)

Signalway Biotechnology

I. Kit Content, Storage and Stability

Content	Storage	10 preps（DP2502）
RNase A	-20℃	1 ml (10 mg/ml）
Buffer P1	4℃	100 ml
Buffer P2	RT	100 ml
Buffer P3	RT	150 ml
Buffer PE	RT	100 ml
Buffer EB	RT	20 ml
Buffer WB	RT	50 ml
		Add 150 ml ethanol before use.
Spin-column AC	RT	10
Collection Tube (50ml）	RT	10

All reagents are stable for 12 months if stored properly.

Reminder:

1. ***Add 150 ml ethanol to Buffer WB before use, mix adequately, and then check the box on the label showing ethanol was added!***
2. ***Add all of the RNase A in the tube into Buffer P1 before starting; the final concentration is 100 μg/ml, then store at 4 ℃.***
3. Buffer P2 may precipitate under low temperature. Incubate at 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.
4. Keep all of the reagents lids tightly caped when not in use to prevent evaporation, oxidation and changes in pH.

II. Principle

The kit applies an improved SDS method to rapidly lyse cells. The DNA is then selectively absorbed onto a silica membrane by a high salt solution. Utilize a series of elution-centrifugation steps to remove cellular metabolites and proteins. Finally we use a low salt elution buffer to elute purified plasmid DNA from the silica membrane.

III. Features

1. Rapid and convenient. No poisonous phenol contained. No need for ethanol precipitation.
2. Multi-elution can ensure high-quality DNA, which is suitable for all kinds of molecular biology experiments like PCR, Southern-blot, restriction enzyme digests, and mammalian transfections.
3. Saving time by avoiding isopropanol precipitation of plasmids and subsequent centrifugation and DNA pallet washing.
4. The yield of plasmid is excellent (up to 2 mg/100-500 ml culture, 200 ml recommended).

IV.Notes

1. All the centrifugation steps can be performed at RT at 4,000-8000 rpm in a traditional centrifuge with a 50 ml rotor.
2. Buffer P2 contains a corrosive compound; please wear latex gloves to avoid contact with skin, eyes and cloth**. If contact occurs then wash with water or physiological saline.**
3. The yield of plasmid is affected by bacteria density and plasmid copy number. The bacteria culture should have a cell density of approximately 10^9 cells/ml or an absorbance of 1-1.5 at 600 nm (A_{600}). For low copy number plasmids or very large plasmids >10 kb, increase the volume of inoculated LB for extracting, with adding extra 50% volume of Buffer P1, P2 and P3.
4. Agarose gel electrophoresis or a UV－spectrometer can be used for measuring the concentration and determining purity of the plasmid. The supercoiled plasmid conformations may display multiple bands at different sizes on your agarose gel. These multiple bands are influenced by culture time and extracting methods.
5. Digest the plasmid to check your DNA size when compared with a DNA marker.
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℃. 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), but be sure to dilute the DNA solution before use because the EDTA will affect down-stream reactions.

V. Procedure

Add 150 ml ethanol to 50 ml Buffer WB!

Add all RNase A to Buffer P1 before starting; the final concentration is 100 μg/ml, then store at 4℃.

Check the solution for precipitate, incubate at 37℃ until clear if necessary!

Please keep Buffer lid tightly caped after use!

1. Harvest culture (100-500 ml, 200 ml recommended) in a 50 ml centrifuge tube by centrifuging at 4,000 rpm for 10-15 minutes, discard supernatant.
2. Add 9 ml Buffer P1, re-suspend cells completely.

   If cells are not re-suspended thoroughly, the cells will not be completely lysed and DNA yield will be decreased.
3. Add 9 ml Buffer P2 and then gently invert the tube 6-10 times to mix thoroughly. Continue until the solution is clear (about 4 minutes).

   Avoid vigorous mixing, and this will result in shearing genomic DNA and lower plasmid purity. Do not let this procedure exceed 5 minutes.
4. Add 14.4 ml Buffer P3， then **invert tube to mix thoroughly** until the flocculated precipitate appears. Incubate at RT for 1 minute. Then centrifuge at 8,000 rpm for 15 minutes (4000~8000 rpm, lower speed needs longer centrifugation time).
5. Add the supernatant from step 4 into Spin-column AC (Add 17 ml to Spin-column each time until all is loaded), centrifuge at 4,000 rpm for 3 minutes, and discard flow-through. Repeat until all supernatant added (4000~8000 rpm, lower speed needs longer centrifugation time).
6. Add 10 ml Buffer PE, centrifuge at 4,000 rpm for 5 minutes, and discard flow-through.

7. Add 10 ml Buffer WB (**make sure the ethanol has been added!),** centrifuge at 4,000 rpm for 3 minutes, and discard flow-through.
8. Repeat step 7.
9. Place the Spin-column AC back on the Collection Tube, centrifuge at 4,000 rpm for 5 minutes.
10. Transfer the Spin-column AC to a clean 50 ml tube, add 1-1.5 ml buffer EB (having been incubated at 65-70℃). Incubate for 1 min at RT, then centrifuge at 4,000 rpm for 3 minutes (4000~8000rpm, lower speed needs longer centrifugation time).

    Buffer EB is Tris-HCl, pH 8.0, and this will not affect down-stream reactions.
11. For maximum efficiency repeat step 10 with 0.5-1 ml buffer EB.

    The volume of elution buffer could be adjusted according to needs. Appropriately reduce elution volume can increase concentration. However, the minimum volume is 0.5 ml. (Less than 0.5 ml will decrease the elution efficiency and the DNA yield.)
12. Transfer the eluted plasmid DNA to a microcentrifuge tube and store purified DNA in -20℃ or apply to down-stream reactions.

VI. Troubleshooting

Problem	Reason	Solutions
Low yield	No antibiotic in the culture, which caused the non- transformants to grow.	Ensure the liquid and solid culture contains the antibiotic.
	Time of culturing is too long; the old cells began to lyse.	Inoculate fresh cells into liquid culture and make sure the culture is not grown for more than 16 hours.
	Use stringent plasmid	Advice using the relaxed plasmid, or increasing volume of treatment.
	The bacteria concentration is too low.	Harvest cells until the [A_{600}] = 2-4.
	Cells not lysed adequately.	Do not harvest too many cells; resuspend cells completely in Buffer P1; then add Buffer P2. The mixture should be sticky and transparent.
	It might not be accurate to measure the concentration using UV －spectrometer,	Use the agarose gel electrophoresis to determine concentration.
	Low elution efficiency	**Please read step10-12 and Notes 6 before starting.**
Contaminated by RNA	RNaseA was not added into Buffer P1; Harvested too many cells; or RNaseA is inactive.	Ensure add RNase A into Buffer P1; If Buffer P1 is more than 3 months, then add more RNase A； Don’t harvest too many cells; Wait a moment for RNase A action after cells are suspended in Buffer P1.

Problem	**Reason**	**Solutions**
DNA digestion inhibition	Eluted silica membrane inhibits digestion.	Centrifuge at 4,000 rpm for 1 minute; Using the supernatant.
	Ethanol remains in spin-column or collection tube bottom.	Make sure step 9 was completed, and wait for 1 minute to do next step
Contaminated with genomic DNA; Nicked plasmid or denatured plasmid band appeared in front of supercoiled one	In the process of isolation, the genomic DNA is broken.	O**verturn to mix thoroughly and gently during the Step 3**. Do not vortex or shake rigorously.
	Time for step 3 is too long.	**Finish step 3 in 5 minutes.**