Note: for laboratory research use only

Endotoxin-free Plasmid DNA Maxi-Preparation Kit (Spin-column)

Cat. #: DP2802 (10 preps)

Signalway Biotechnology

Ⅰ.Kit Content, Storage and Stability

Content	Storage	200 preps (DP1003)
RNase A	-20 ℃	500 µl(10 mg/ml）
Buffer P1	4 ℃	100 ml
Buffer P2	RT	100 ml
Buffer P3	RT	150 ml
Buffer E-WB	RT	100 ml
Buffer EB	RT	20 ml
Buffer WB	RT	50 ml
		Add 150 ml ethanol before first use
Spin Column AC	RT	10
Collection Tube (2ml)	RT	10
Purification-column ED	RT	10

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

Reminder:

1 · ***Add 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 100µg/ml; store Buffer P1 at 4 ℃.***

3 · Buffer P2 may precipitate under low temperature. Incubate buffer P2 at 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.

4 · Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and change in pH.

Ⅱ.Principle:

The kit applies the improved SDS method to rapidly lyse cells. Plasmid DNA selectively adsorbs on silica membrane in high salt solution. A serial of elution-centrifugation steps will remove cellular metabolites and proteins (Endotoxin is removed by the purification-column). Plasmid DNA is eluted out in a low salt and high pH buffer.

Ⅲ.Features:

1・Rapid and convenient. No poisonous phenol contained. No need for ethanol precipitation.

2・Multiple washing-elutions yield DNA in high purity, which can be applied to all kinds of molecular biology experiments such as PCR, Southern-blot, restriction enzyme digests, and mammalian transfections.

3・The yield of plasmid is 0.5-1.5 mg/200 ml culture.

4・The specific reagent is included for removing endotoxin, which ensures the level of endotoxin < 0.1 EU/ μg.

Ⅳ.Notes

Read this section before your experiment.

1. All centrifugation steps can be performed at room temperature.
2. Buffer P2 contains a corrosive compound; please wear latex gloves to avoid contact with skin, eyes and clothes**. If contact occurs, wash with water or physiological saline.**
3. The yield of plasmid is affected with culture concentration and copy number. The bacteria culture should have an approximate 10^9 cells/ml cell density or 1-1.5 absorbance at 600 nm (A_{600}). For high copy plasmids, picking a single colony from a freshly streaked plate, inoculating in 200 ml LB medium with appropriate antibiotic, shaking over night at 37°C, the yield of plasmid may achieve 30 μg. For low copy plasmids or size>10 kb plasmids, we recommend collecting 300 ml

overnight culture and using 50% extra volume of buffer P1, P2 and P3.

4. Agarose gel electrophoresis or UV－spectrometer can be used for detecting the concentration and purity of the plasmid. The supercoiled plasmid conformation 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 by comparing with a DNA marker.
6. There is no EDTA in Buffer EB, which should not affect down-stream reactions. If interference occurs, use sterile water (pH >7.5) to elute and store DNA at -20℃. Low pH will decrease the elution efficiency. For long-term storage, elute plasmid in TE (10mM Tris-HCl, 1mM EDTA, pH 8.0) and be sure to dilute the DNA solution before use because the EDTA will affect down-stream reactions.

Ⅴ.Procedure

Before Starting

- ***Add the all RNase A to Buffer P1 before use; Store the P1/RNase A mixture at 4℃.***
- ***Dilute Buffer WB with 150ml absolute ethanol, vortex adequately, and check the box on the bottle!***

1・Harvest culture (100-500 ml, 200 ml recommended) in a 500 ml centrifuge tube by centrifuging at 8,000 rpm for 10-15 minutes and discard supernatant.

2・Add 9 ml Buffer P1 and 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 invert the tube 6-10 times to mix thoroughly but gently. Continue until the solution is clear (about 4 minutes).

Avoid vigorous mixing that will result in shearing genomic DNA and reduction of plasmid purity. Do not let this procedure exceed 5 minutes.

4・Add 14.4 ml Buffer P3，then invert tube to mix thoroughly and gently until the

flocculated precipitate appears. Incubate at RT for 1 minute, then centrifuge at 8,000 rpm for 15 minutes.

5・Add the supernatant from step 4 into Spin-column AC (once 17 ml), centrifuge at 8,000 rpm for 3 min, and discard flow-through. Repeat until all supernatant added.

6・Add 10 ml Buffer E-WB, centrifuge at 8,000 rpm for 5 minutes, and discard flow-through.

7・Add 10 ml Buffer WB **(make sure the ethanol has been added!),** centrifuge at 8,000 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 (preheated to 65-70℃). Incubate for 1 min at RT, then centrifuge at 4,000 rpm for 3 minutes. (4000~8000 rpm, lower speed needs longer centrifugation time)
    Buffer EB is Tris-HCl (pH 8.0), which will not affect down-stream reactions.

11. Transfer the filtrate from step 10 to the Purification-column ED (once 750 μl). Centrifuge at 13,000 rpm for 1 min, and collect filtrate (containing plasmid DNA).

12. Store DNA in -20℃ or apply to down-stream reactions.

VI. Troubleshooting

Problem	Reason	Solutions
Low yield	No antibiotic in culture, which cause over-growth of the non-transformed cells.	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. The time of culturing is not over 16 hours.
	Use stringent plasmid	Advise using the relaxed plasmid, or increasing volume of treatment.
	The concentration of cells is too low.	Harvest cells until the [A_{600}] =2-4.
	Cells not lysed adequately.	Avoid using too many cells; suspend cells completely in Buffer P1; After adding Buffer P2, the mixture should be sticky and transparent.
	It may not be accurate to measure the concentration by UV－spectrometer.	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 ethanol before use.**
	There is too much ethanol in the elution buffer; and the DNA float out the lanes before electrophoresis	Ensure proceed step10, and no ethanol remains; Increase the volume of loading buffer