Note: for laboratory research use only

High-purity Plasmid DNA Mini-Preparation Kit (Spin-column)

Cat. #: DP1002 (200 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℃	50 ml
Buffer P2	RT	50 ml
Buffer P3	RT	80 ml
Buffer PE	RT	100 ml
Buffer WB	RT	50 ml
		Add 150 ml ethanol before first use
Buffer EB	RT	20 ml
Spin Column AC	RT	200 pcs
Collection Tube （2ml）	RT	200 pcs

All reagents are stable for 18 months at RT if 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 100 μg/ml, then store at 4 ℃.***
3. Buffer P2 may precipitate under low temperature. Incubate to 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:

This kit is based on a modified alkaline-SDS lysis procedure, followed by binding of plasmid DNA to silica-membrane under appropriate high-salt and low pH conditions. Proteins and low molecular weight impurities are removed by Buffer PE

and WB. Utilize a series of elution-centrifugation steps to remove cellular metabolites and proteins. Then plasmid DNA is eluted from in a low salt and high pH buffer.

Ⅲ. Features:

1. Rapid and convenient. No poisonous phenol contained. No need for ethanol precipitation. Multi-elution can ensure high-purified DNA, which can be applied to all kinds of molecular biology experiments such as PCR, Southern-blot, restriction enzyme digests, and mammalian transfections.
2. Unique content can effectively remove the nuclease and even apply to rich-nuclease stains, such as JM and HB101.
3. The yield of plasmid is excellent (up to 30 μg/1-5 ml culture, 2-4 ml recommended).

Ⅳ. Notes

Read this section before your experiment.

1. All the centrifugation can be performed at room temperature.
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 600nm (A_{600}). For high copy plasmids, picking a single colony from a freshly streaked selective plate, inoculating in 2-4 ml LB medium containing the appropriate selective 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 5-10ml overnight culture and using 50% extra 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). 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 150 ml absolute ethanol, vortex adequately, then mark the check box, avoid multi-adding!***

1. Harvest 2-4 ml overnight culture fluid, centrifugation at 9,000 rpm for 30 seconds. Collect bacterial pellet, discard the supernatant.
2. Resuspend the bacterial pellet by adding 250 µl Buffer P1. Complete resuspension (no visible cell clumps) of cell pellet is vital for obtaining good yields.
3. Add 250 µl Buffer P2 and gently mix by inverting and rotating tube 6-10 times to obtain a clear lysate.

   Avoid vigorous mixing, which will result in shearing genomic DNA and lower plasmid purity. Do not let this procedure exceed 5 minutes.
4. Add 400 µl Buffer P3 and then **invert tube to mix thoroughly and gently until** the flocculated precipitate appears. Incubate at room temperature for 1 minute. Centrifuge at 13,000 rpm for 10 minutes at room temperature.
5. Add the clear supernatant carefully into Spin-column AC. Centrifuge at 13,000 rpm for 1 minute. Discard the flow-through liquid.
6. Add 500 µl Buffer PE. Centrifuge for 30-60 seconds at 13,000 rpm. Discard the flow-through.
7. Add 500 µl Buffer WB. Centrifuge for 30-60 seconds at 13,000 rpm. Discard the flow-through.

Buffer WB must be diluted with 150 ml absolute ethanol before first use.

8. Repeat step 7.
9. Place the Spin-column AC back on the Collection Tube, centrifuge at 13,000 rpm for 2 minutes.
10. Transfer the Spin-column AC to a clean1.5 ml microcentrifuge tube, add 60-100 µl Buffer EB (pre-warmed at 65℃−70℃ before use) directly onto the silica-membrane. Incubate 1 minute at room temperature. Centrifuge at 13,000 rpm at 1 minute.
11. For maximum efficiency repeat step 10 with 50 µl 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 50 µl (Less than 50 µl will decrease the elution efficiency and the DNA yield).
12. Keep DNA at -20℃ or apply to down-stream reactions.

Ⅶ. Troubleshooting

Problems	Causes	Advices
Low yield	No antibiotic in culture, which cause the non-transformants overgrowth.	Ensure the liquid and solid culture contains the antibiotic.
	Bacteria are overgrown	Do not incubate cultures for more than 16 hours at 37℃.
	Low copy number of plasmid used	Use the relaxed plasmid, or increase culture volume.
	The bacteria concentration is too low.	Harvest cells until the [A_{600}] =2-4.
	Poor cell lysis	Do not harvest too many cells; Make sure to thoughly vortex cell suspension in Buffer P1. The mixture should be sticky and transparent after adding the Buffer P2.
	It might not be accurate to measure the concentration using UV－spectrometer,	Using the gel electrophoresis to determine concentration.
	Low elution efficiency	**Please read step 10-12 and Notes 6 before use**

Problems	Causes	Advices
No DNA eluted	Buffer WB not diluted with absolute ethanol	**Add 150 ml ethanol before use**
	Ethanol remains in spin column; or the DNA float out the lanes before electrophoresis	Ensure to proceed step 9, and no ethanol remains; Increasing the volume of loading buffer.

DNA digestion inhibition	Silica membrane eluted	Centrifuge at 13,000 rpm for 1 minute to spin down the silica membrane debris. Using the supernatant.
	ethanol remained in spin column	Ensure to proceed step 9 and air-dry for a few minutes
The DNA degrades, or no DNA	The activity of nuclease is too high	Ensure to proceed step 7 to remove nuclease
High molecular weight DNA contamination	In the process of lyses, the genomic DNA is broken.	Perform step 3 and **mix thoroughly and gently**. Do not vortex or mix vigorously after adding solution P2.
Nicked plasmid or having the denatured plasmid band appeared in front of supercoiled one	Time for step 3 is too long.	**Complete step 3 in 5 minutes.**
The product contaminated by RNA	RNaseA not add into Buffer P1; Too much cells treated; 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 treat too much cells ； Wait a moment for RNase A action after cells are suspended in Buffer P1.