Note: for laboratory research use only

High-Purity Plasmid DNA Mini-Preparation Kit

(Spin-column)

Cat. #: **DP1001 (50 preps)**
DP1002 (100 preps)
DP1003 (200 preps)

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Ⅰ.Kit Content, Storage and Stability

Content	Storage	50 preps （DP1001）	100 preps (DP1002)	200 preps (DP1003）
RNase A	－20℃	150μl(10mg/ml)	300μl(10mg/ml)	500μl(10mg/ml）
Buffer P1	4℃	15 ml	30 ml	50 ml
Buffer P2	RT	15 ml	30 ml	50 ml
Buffer P3	RT	20 ml	40 ml	80 ml
Buffer PE	RT	27 ml	50 ml	100 ml
Buffer WB	RT	15 ml	25 ml	50 ml
		Add ration ethanol before first use		
Buffer EB	RT	15 ml	15 ml	20 ml
Spin Column AC	RT	50 pcs	100 pcs	200 pcs
Collection Tube（2ml）	RT	50 pcs	100 pcs	200 pcs

All reagents are stable for 18 months at RT, when stored properly.

Notes:

1. If RNaseA is inactive, RNA will contaminate the plasmid. Add additional RNaseA to Buffer P1.
2. Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C
3. Please ensure the bottles of buffer tightly capped when not in use to prevent reagents evaporating, oxidation and pH changing.
4. Dilute Buffer WB with three volume absolute ethanol before start.

Ⅱ.Principle:

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

Buffer PE and WB. Then plasmid DNA is eluted from in a low salt and high pH buffer. .

Ⅲ.Features:

1. Rapid and convenient. Do not contain poisonous phenol etc and not need carrying ethanol precipitation. Multi-elution can ensure high-purified DNA, which can be applied to all kinds of molecular experiments such as PCR，Southern-blot and digestions directly.
2. The silica membranes in the spin-column come from the world-famous company.
3. Unique content can effectively remove the nuclease, even apply to rich-nuclease stains of JM and HB101,

Ⅳ.Notes

Please read this section before your experiment.

1. All the centrifugation can be performed at room temperature.
2. Buffer P3 includes the stimulating compound. Please wear latex gloves, avoiding skin, eyes and cloth to be contaminated. **If that, please use water or physiological saline washing.**
3. The yield of plasmid is related with concentration of liquid culture and copy number. For high copy plasmids, picking a single colony from a freshly streaked selective plate, inoculating in 1.5-4.5 ml LB medium containing the appropriate selective antibiotic, shaking over night at 37°C, the yield of plasmid may achieve 20μg.For low copy plasmids or size>10kb plasmids, we recommend collecting 5-10ml overnight culture and scaling up volumes of buffer P1, P2 and P3.
4. DNA concentration and quality can be determined by UV and agarose gel electrophoresis. $1OD_{260}$ may be 50μg/ml DNA. Typically, the majority of the eluted DNA is in monomeric supercoil form, sometimes they may display different types, single or two even more bands in agarose gel electrophoresis because of influenced by culture time and operations of extracting.
5. If want to know the size of the plasmid, please make the restriction endonuclease

digestion to get the exact size compared with the DNA Marker.

6. No EDTA in Buffer EB, which will have no influence on down-stream reactions. Also you can use water when eluting, but please ensure pH>7.5 and store at -20℃. If for long-term storage, dissolve plasmid in TE（10mM Tris-HCl， 1mM EDTA，pH 8.0）. Because EDTA will affect the down-stream reactions, dilute the solution before use.

Ⅴ.Procedure

Before Starting

- *Add the all the provided RNase A to Buffer P1 before use, to give a final concentration of 100μg/ml. Store the P1/RNase A mixture at 4℃.*
- *Dilute BufferWB with three times volume of absolute ethanol, vortex adequately, then mark the check box, avoid multi-adding!*

1. Harvest 1.5-4.5ml overnight culture fluid, centrifugation at 9,000rpm for 30s. Collect bacterial pellet, discard the supernatant.
2. Resuspend the bacterial pellet by adding 250μl Buffer P1, and vortex. Complete resuspension (no visible cell clumps) of cell pellet is vital for obtaining good yields.
3. Add 250μl Buffer P2 and gently mix by inverting and rotating tube 6-10 times to obtain a clear lysate. Avoid vigorous mixing as this will resulting in shearing chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 min as this will damage plasmid.
4. Add 400μl Buffer P3 and immediately mix by inverting and rotating tube 6-10 times. Incubate at room temperature for 5min. Centrifuge at 13,000rpm for 10min at room temperature.
5. Add the clear supernatant carefully into Spin-column AC. Centrifuge at 13,000 for 1min. Discard the flow-through liquid.
6. Add 500μl Buffer PE. Centrifuge for 30-60s at 13,000rpm. Discard the flow-through.
7. Add 500μl Buffer WB. Centrifuge for 30-60s at 13,000rpm. Discard the flow-through.

   Note: Buffer WB must be diluted with absolute ethanol before first use.
8. Centrifuge the empty column at 13,000rpm for 2 min. Air-dry for 3-5 min at room temperature.

9. Transfer the Spin-column AC to a clean1.5ml microcentrifuge tube, add 60-100µl Buffer EB (water bath in 65-℃70℃ before use) directly onto the silica-membrane. Incubate 1 min at room temperature. Centrifuge at 13,000rpm at 1 min.

   The volume of elution buffer could be adjusted according to needs. Appropriately reduce elution volume can increase concentration. But the minimum volume is 50µl, too less will decrease the elution efficiency and the DNA yield.

10. Keep DNA at 2-8℃ (-20℃ for long-term storage).

Ⅶ.Troubleshooting

Problems	Causes	Advices
Low yield	No antibiotic in culture, which cause the non-transformants overgrowth.	Ensure the liquid and solid culture contain the antibiotic.
	Bacterial clone is overgrown	Do not incubate cultures for more than 16 hr at 37℃.
	Low copy number of plasmid used	Use the relaxed plasmid, or increase culture volume.
	The concentration is too low, not enough	Harvest cells until the [A_{600}]=2--4.
	Poor cell lysis	Please don't treat too much cells; Make sure to vortex cell suspension to completely in Buffer P1. the mixture should be sticky and transparence after adding the Buffer P2.
	By UV-Spectrometer, the concentration usually is on the high side	Using the gel electrophoresis to determine concentration.
	Low elution efficiency	**Please read step 9 and Notes 6 before use**

No DNA eluted	Buffer WB not diluted with absolute ethanol	**Add ration ethanol before first use**
	Too much ethanol in the elution buffer; or the DNA float out the lanes before Electrophoresis	Ensure have had step10, and no ethanol remains; Increasing the volume of loading buffer
DNA digestion inhibition	Silica membrane eluted	Centrifuge at 13,000 rpm for 1 minute, carefully using the supernatant
	ethanol remained in spin column	Ensure do step 9 and air-dry for a few minutes
The DNA degrades, or no DNA	The activity of nuclease is too high	Ensure do step7 to remove nuclease
High molecular weight DNA contamination of product.	In the process of lyses, the genomic DNA is broken.	Do step3 and **mix thoroughly and gently**. Do not vortex and mix aggressively after adding solutionP2.
Nicked plasmid or having the denatured plasmid band appeared in front of supercoiled one	It is too long time for step3	Do not allow the lysis reaction to proceed more than 5 min.
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 ; when cells are suspended in Buffer P1, can wait a moment for RNasse A action