Note: for laboratory research use only.

Endotoxin-free Plasmid DNA Mini-preparation Kit (Spin-column)

Cat. # DP2601 (20 preps)
DP2602 (50 preps)

Signalway Biotechnology

I. Kit Content Storage and Stability

Content	Storage	20 preps (DP2601)	50 preps (DP2602)
RNase A	-20°C	60 μl (10 mg/ml)	150 μl (10 mg/ml）
Buffer P1	4°C	6 ml	15 ml
Buffer P2	RT	6 ml	15 ml
Buffer P3	RT	8 ml	20 ml
Buffer E-WB	RT	11ml	27 ml
Buffer WB	RT	6 ml	15 ml
		Add 24 ml (20 preps) or 60 ml (50 preps) ethanol before use.	
Buffer EB	RT	15 ml	20 ml
Spin-column ED	RT	20	50
Collection Tube (2 ml)	RT	20	50

All reagents are stable for 18 months when stored properly.

Notes:

1. **Please add ethanol into Buffer WB before starting, 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 few minutes until clear, then cool down to RT for use.
4. Please keep all reagents' lid tightly caped when not in use to prevent reagents evaporating, oxidation, and pH change.

II. Principle

The kit applies the improved SDS method to rapidly lyses cells, the endotoxin in crude extracting will be selectively precipitated by specific agent and removed by centrifugation. Then DNA selectively absorbs on silica membrane in high salt solution. 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 is not used in this kit. Multi-elution can ensure high-quality DNA, which are suitable for all kinds of molecular experiments such as transfection, PCR, Southern-blot, and enzyme digestion directly.
2. The unique solutions can effectively remove the nuclease of bacteria including nuclease-rich JM and HB101 strains.
3. The specific agent is prepared for removing endotoxin, which ensures the level of endotoxin <0.1 EU/μg.

IV.Notes

1. **All the centrifugation steps can be performed at RT** and 13,000 rpm in a traditional centrifuge.
2. Buffer P3 contains the stimulating compound; please wear latex gloves to avoid skin, eyes, and cloth to be contaminated. **Please wash with water or physiological saline when contact occurs.**
3. **The yield of plasmid depends on culture concentration and plasmid copy number.** For relaxed plasmid, just inoculate cells into 1.5-4.5 ml LB and culture overnight; for stringent plasmid or the plasmid size >10 kb, increase the volume of LB for extracting, with increasing volume of Buffer P1, P2 and P3 in a proper ratio.
4. The agarose gel electrophoresis and 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 affected by culture time and extracting methods.

5. Please digest plasmid to check the exact size of plasmid by comparing with DNA markers.
6. There is no EDTA in Buffer EB, which will not affect down-stream reactions. If interference occurs, use sterile water (pH >7.5) to elute and store DNA at -20°C. 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.
7. The plasmid yield will be decreased (around 10%) due to endotoxin-removal reagent used in the procedure.

V. Procedure

Add 24 ml ethanol into 6 ml Buffer WB or 60 ml ethanol into 15 ml Buffer WB before starting!

Add all RNase A in the tube into Buffer P1 before starting; the final concentration is 100 mg/ml, then store at 4°C.

Put Buffer P3 on ice before starting!

1. Harvest cells in a microcentrifuge tube by centrifuging at 9,000 rpm for 30 sec. Discard supernatant.
2. Add 250 µl Buffer P1 and suspend cells completely.

   Incomplete suspension will affect lyses and decrease plasmid yield.
3. Add 250 µl Buffer P2, and then gently overturn tube 4-7 times to mix thoroughly until sample become clear.

   Complete this procedure in 5 minutes and gently overturn tube to avoid breaking plasmid.
4. Add 350 µl Buffer P3，then **overturn to mix thoroughly and gently** until the thick flocculated precipitate appear, and incubate on ice. Centrifuge at 13,000 rpm for 10 minutes.

Overturn to mix thoroughly immediately after adding Buffer P3 to avoid SDS precipitation. If still have precipitation on surface of solution after centrifuge, please centrifuge again.

5. Transfer the supernatant into the Spin-column ED (on collection tube). Centrifuge at 13,000 rpm for 1 minute, and discard flow-through.

6. Add 500 µl Buffer E-WB，centrifuge at 13,000 rpm for 30-60 sec, and discard flow-through.
7. Add 700 µl Buffer WB (**please check if ethanol added!**)，centrifuge at 13,000 rpm for 30 -60 sec, and discard flow-through.
8. Add 500 µl Buffer WB，centrifuge at 13,000 rpm for 30-60 sec, and discard flow-through.
9. Centrifuge Spin-column ED at 13,000 rpm for 2 minutes, and place at RT for 5 minutes to remove remaining ethanol.
10. Transfer Spin-column ED to a clean tube, add 100 µl Buffer EB (preheated to 65-70°C), stay at RT for 3 minutes, and centrifuge at 13,000 rpm for 1 minute. Add the flow-through back into the Spin-column AC, stay at RT for 3 minutes, and then centrifuge at 13,000 rpm for 1 minute.

    Reducing elution volume will increase the purified DNA concentration. However, if the elution volume is less than 50 µl, elution efficiency and DNA yield can be affected.
11. Keep DNA at 2-8°C, or at -20°C for long-term storage.

VI. Troubleshooting

Problem	Reason	Solutions
Low yield	No antibiotic in culture, which cause over-growth of the non-transformed cells.	Ensure that the liquid and solid cultures contain the antibiotic.
	Time of culturing is too long; the old cells begin lyse.	Inoculate fresh cells into liquid culture. The time of culturing should not be over 16 hours.
	Use stringent plasmid	Advice 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.	Please don't treat too many cells; suspend cells completely in Buffer P1； After adding Buffer P2, the mixture should be sticky and transparent.
	Low elution efficiency	**Please read step 10-12and Notes 6 before starting.**
No product	Ethanol not added to Buffer WB	**Add ethanol before use.**
	There is too much ethanol in the final solution.	Ensure to perform step 9.
DNA digestion inhibition	Eluted silica membrane inhibits digestion.	Centrifuge at 13,000 rpm for 1 min, carefully transfer the supernatant to remove contaminant.
DNA digestion inhibition Contaminated with genomic DNA	Ethanol remains in spin-column or collection tube bottom.	Ensure to perform step 9.
	In the process of isolation, the genomic DNA is broken.	In step 3, **overturn to mix thoroughly and gently**. Do not vortex and shake rigorously.
Nicked plasmid or denatured plasmid band appeared in front of supercoiled one	Time for step 3 is too long.	**Complete step 3 in 5 minutes.**
Contaminated by RNA	RNase A not added into Buffer P1; Too many cells harvested; RNaseA is inactive.	Add RNase A into Buffer P1; If Buffer P1 has been stored over 3 months, add new RNase A into it. Don't harvest too many cells; when cells are suspended in Buffer P1, wait a moment for RNase A reaction.