*Note: for laboratory research use only.*

# Endotoxin-free Plasmid DNA Mini-preparation Kit

# (Spin-column)

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

**ABigen corporation**

www.abigen.com Tel:+86-01-13683383835 E-mail: info@abigen.com

## I. Kit Content Storage and Stability

| Content | Storage | 20 preps （DP2601） | 50 preps (DP2602） |
|---|---|---|---|
| RNase A | -20℃ | 60μl (10mg/ml) | 150μl (10mg/ml） |
| Buffer P1 | 4℃ | 6ml | 15ml |
| Buffer P2 | RT | 6ml | 15ml |
| Buffer P3 | RT | 8 ml | 20ml |
| Buffer P4 | RT | 9ml | 22ml |
| Buffer PE | RT | 11ml | 27ml |
| Buffer WB | RT | 6ml | 15ml |
| | | ***Add the ration ethanol before use.*** | |
| Endotoxin-removal Solution | 4 (for a month); -20 (for long term) | 2ml | 5ml |
| Buffer EB | RT | 15ml | 20ml |
| Spin-column AC | RT | 20 | 50 |
| Collection Tube （2ml） | RT 20 | | 50 |

***All reagents are stable for 18 months when stored properly.***

### Notes:

1. **Please add the ration 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 100mg/ml, then store at 4 .**℃
3. Buffer P2 may precipitate under low temperature. Incubate to 37 for a moment **until clear**, then cool down to RT for use.
4. Please keep all reagents' lid tightly when not in use to prevent reagents evaporating, oxidation and pH change.

## II. Principle

The kit applies the improved SDS method to rapidly lyses cell, the endotoxin in crude extracting will be selectively combined by specific agent and removed by centrifugation. Then DNA selectively absorbs on silica membrane in high salt solution. Take 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 etc is not used in this kit. Multi-elution can ensure high-quality DNA, which are suitable for all kinds of molecular experiments such as PCR, Southern-blot and enzyme digestion directly.
2. The silica membranes in the spin-column come from the world-famous company.
3. The unique solutions that can effectively remove the nuclease, even the stains of JM and HB101, which are rich in nuclease.
4. 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 traditional centrifuge, for example Eppendorf 5415C and the similar.
2. Buffer P3 contains the stimulating compound; please wear latex gloves to avoid skin, eyes and cloth to be contaminated. **If that, please wash with water or physiological saline.**
3. **The yield of plasmid depends on culture concentration and plasmid copy number.** So for relaxed plasmid, just inoculate the cells into 1.5-4.5ml LB and culture over-night; for stringent plasmid or the size >10kb, increase the volume of inoculated LB for extracting, with rational increasing volume of Buffer P1, P2 and

P3.

4. The agarose gel electrophoresis and UV－spectrometer can be used for detecting the concentration and purity of the plasmid. 1$OD_{260}$ may be 50μg/ml DNA. The supercoiled plasmid conformations may display different types, single or two even more bands in agarose gel electrophoresis, because their types are influenced by culture time and operations of extracting.
5. Please digest plasmid to check the exact size of plasmid when compared with DNA marker.
6. There is no EDTA in Buffer EB, which will not affect down-stream reactions. Also use water to elute DNA, but please ensure pH>7.5, and store at -20℃. Low pH will decrease the elution efficiency. For long-term storage, dissolve plasmid in TE (10mM Tris-HCl, 1mM EDTA, pH 8.0). But please dilute the DNA solution before use because EDTA will affect the down-stream reactions.
7. The plasmid yield will be decreasing (around 10%) due to endotoxin-removal reagent used in the procedure.

## V. Procedure

***Please add 24ml ethanol into 6ml Buffer WB or 60ml ethanol into 15ml Buffer WB before starting!***

***Please add all RNase A in the tube into Buffer P1 before starting; the final concentration is 100mg/ml, then store at 4 .℃***

***Please put Buffer P3 on ice before starting!***

1. Harvest cells in a microcentrifuge tube by centrifuging at 9,000rpm for30sec. Discard supernatant as far as possible.

   **For more cells, centrifuge more culture in the same tube.**
2. Add 250μl Buffer P1 and suspend cells completely.

   **If not, will affect lyses and seriously decrease products**
3. Add 250μl Buffer P2, and then overturn 4-7 times to mix thoroughly and gently

until clear.

***Please overturn to mix thoroughly and complete this procedure in 5 minutes, avoiding breaking plasmid.***

4. Add 350μl Buffer P3，then **overturn to mix thoroughly and gently until** the thick flocculated precipitate appear, and incubate on ice. Then centrifuge at 13,000rpm for 10min and transfer the supernatant into a new pre-cooling tube.

   ***Please overturn to mix thoroughly immediately after add Buffer P3 to avoid SDS precipitation. If still have precipitation on surface of solution, please centrifuge again.***

5. Add Endotoxin-removal Solution (about 1/10 volume of supernatant) into the tube then **overturn 4-7 times to mix thoroughly and gently,** and incubate on ice for 20min. Please overturn every once in a while.

   ***Once Endotoxin-removal Solution added, the mixture will become cloudy. Don't worry! It will be clear again after incubate on ice for a moment.***

6. Incubate to 37℃ until the solution will become cloudy again. Then overturn several times and incubate 20-30min at 37℃, until the solution appears the obvious two phases.

7. Centrifuge at 12,000rpm for 5min at 30℃. Please keep temperature above 25 ℃. **Solution will separate into two phases.** If not, please repeat the step 6 and 7.

   **DNA is in the upper phase and endotoxin in the lower phase.**

8. Transfer the upper phase into a new tube. (Don't take any lower phase solution, which is rich in endotoxin).

9. ***Add Buffer PB (around 1/2 volume of upper phase) into the tube,*** *then* ***overturn to mix thoroughly and gently***

10. Transfer the mixture (once 700μl) into Spin-column AC (on the Collection Tube), centrifuge at 13,000rpm for 30-60sec, and discards flow-through.

    **Optional step**: Add 500μl Buffer PE, centrifuge at 13,000rpm for 30-60sec, and discard flow-through.

***This optional step is needed for strains of JM, HB101 and so on, which are rich***

***in nuclease.***

11. Add 700µl Buffer WB（**please check if ethanol added!**），centrifuge at 13,000 rpm for 30 -60sec, and discard flow-through.
12. Add 500µl Buffer WB，centrifuge at 13,000 rpm for 30-60sec, and discard flow-through.
13. Centrifuge Spin-column AC at 13,000 rpm for 2min, and place at RT for 5min to remove remain ethanol.
14. Transfer Spin-column AC to a clean tube, add 100µl Buffer EB (having been incubation at 65-70 )℃ , stay at RT for 3-5min, and centrifuge at 13,000 rpm for 1min. Add the flow-through back into the Spin-column AC, stay at RT for 3-5min, and then centrifuge at 13,000 rpm for 1 min.

    ***Please reduce elution volume to increase the purified DNA concentration. But if the elution volume is less than 50 µl, elution efficiency and DNA yield can be affected.***
15. Keep DNA at 2-8 , please at -20℃ for long-term storage.

## Troubleshooting

| Problem | Reason | Solutions |
|---|---|---|
| Low yield | No antibiotic in culture, which cause the non-transformants over-growth. | 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 and the time of culturing is not over 16 hours. |
| | Use stringent plasmid | Advice using the relaxed plasmid, or increasing volume of treatment. |
| | The concentration is too low or not enough. | Harvest cells until the [$A_{600}$] =2-4. |
| | Cells not lysed adequately. | Please don't treat too much cells; suspend cells completely in Buffer P1； After add Buffer P2, the mixture should be sticky and transparent. |
| | By UV－spectrometer, the concentration usually is on the high side. | Use the agarose gel electrophoresis to determine concentration. |
| | Low elution efficiency | **Please read step15 and Notes 6 before starting.** |
| No product | Ethanol not added to Buffer WB | **Add the ration ethanol before use.** |
| | There is too much ethanol in the elution buffer; and the DNA float out the lanes before electrophoresis | Ensure have had step14, and no ethanol remains; Increase the volume of loading buffer |

*To be continued*

*Continued*

| Problem | Reason | Solutions |
|---|---|---|
| DNA digestion inhibition | Eluted silica membrane inhibits digestion. | Centrifuge at 13,000 rpm for 1 minute, carefully take the supernatant to remove contaminant. |
| | Ethanol remains in spin-column or collection tube bottom. | Ensure do step 14, and wait for a moment to do next step |
| The DNA degrades or no DNA | The activity of nuclease is too high. | Ensure do step11 to remove nuclease. |
| Contaminated with genomic DNA | In the process of isolation, the genomic DNA is broken. | Do step 3 and **overturn to mix thoroughly and gently**. Do not vortex and shake rigorously. **Please do step 3 in 5 min.** |
| Nicked plasmid or denatured plasmid band appeared in front of supercoiled one | Time for step 3 is too long. | |
| Contaminated by RNA | RNaseA not added into Buffer P1; Too much cells treated; or RNaseA is inactive. | Ensure add RNase A into Buffer P1; If Buffer P1have been stored over 3 months, then add new RNase A into it; Don't treat too much cells; when cells are suspended in Buffer P1, please wait a moment for RNase A reaction. |