

Bacterial DNA Extraction Kit

(Spin-column)

The Kit provides a fast way to isolate pure DNA from all kinds of bacteria

For laboratory research use only

DP2001 **50 preps**

Kit Content, Storage, and Stability

Content	Storage	50 preps
Buffer RB	RT	22 ml
Buffer CB	RT	11 ml
Buffer IR	RT	27 ml
Buffer WB	RT	15 ml
	Add ration ethanol before use	
Buffer EB	RT	15 ml
Isopropanol	RT	7 ml
Proteinase K (20 mg/ml)	-20°C	20 mg (powder)
Spin-column AC	RT	50
Collection Tube (2 ml)	RT	50

All reagents are stable for 12 months when stored properly.

Notes

Please add 60ml ethanol to 15ml Buffer WB before first time, vortex adequately, and then check it to avoid multi-adding!

Buffer CB or IR may precipitate under low temperature, incubate them at 37 °C water-bath for a moment **until clear**, then cool down to RT for use.

Proteinase K is provided in freeze-dried powder for activity and transportation. Centrifuge a few seconds and **add 1ml sterile water to the tube**. Because frozen and melt repeatedly may affect enzyme activity, please aliquot and store under -20 °C.

Please cap all reagents bottles tightly after use to prevent reagents from evaporating, oxidation and pH changing.

Principle

The Kit applies the unique binding buffer/ Proteinase K to rapidly lyse cells and inactivate cellular nucleases, then DNA is selectively absorbed on silica membrane in high salt solution, after that serial of elution - centrifugation steps remove cellular metabolite and proteins. Finally low salt elution elutes purified genomic DNA from silica membrane

Features

1. Poisonous phenol etc not used.
2. Procedure is simple and fast, single sample can be completed in 30 min.
3. Multi-elution can ensure high-purified DNA, the typical ratio of OD260/OD280 is 1.7~1.9, and the average length up to 30Kb-50kb, which can be applied for PCR, Southern-blot and digestions directly.

Notes

1. **All the centrifugation steps can be performed at RT** and 13,000 rpm in traditional centrifuge.
2. Before use, please set water-bath at 70 °C
3. Buffer CB and IR contains the stimulating compound, please wear latex gloves, and avoid skin, eyes and cloth to be contaminated. If that, please use water or physiological saline washing.

Procedure

**REMEMBER - Please dilute 15 ml Buffer WB with 60 ml absolute ethanol.
For Gram – positive bacteria prepare the lysozyme or lysostaphin by yourself.**

1. Harvest cells (maximum 2×10^9 cells) in a microcentrifuge tube by centrifuging at 10,000 rpm for 30 sec. Discard supernatant as soon as possible.
Initial sample amount depends on microbeing species and growth density, but the maximum absorption of spin-column membrane is up to 25µg genomic DNA, so large initial sample amount will decrease DNA yield.
2. Add 200 µl buffer RB to suspend and wash cells. Centrifuge at 10,000 rpm for 30 sec, discard supernatant, and then resuspend pellet in 200 µl Buffer RB.
3. **For gram-negative bacteria** (alternative): add 5µl lysozyme (10mg/ml in 10 mM Tris-HCl, pH 8.0), overturn to mix thoroughly, and incubate at 37°C water-bath for 15 min.
For gram-positive bacteria: add 50µl-100µl lysozyme (10mg/ml in 10 mM Tris-HCl, pH 8.0) overturn to mix thoroughly, incubate at 37°C water-bath for 30-60 min. centrifuge at 10,000 rpm 2 min, discard supernatant, and then resuspend pellet in 200 µl Buffer RB.
For most gram-positive bacteria: *Bacillus subtilis*, *Micrococcus luteus*, *Arthrobacter luteus*, *Nocardia otitidiscaviarum*, *Rhodococcus rhodochrous*, and *Brevibacterium albidium*, lysozyme can lyse cells completely. But for some bacteria such as *Staphylococcus*, must add 25µl lysozyme (10mg/ml) and 25µl lysostaphin (10mg/ml) to disrupt cells.
4. Alternative step: add 20µl RNase A (25 mg/ml) solution, vortex to mix thoroughly, stand at RT for 5-10 min to remove RNA.
5. Add 200µl buffer CB, then overturn to mix thoroughly, add 20µl Proteinase K (20mg/ml), mix thoroughly, incubate at 70°C water-bath for 10 min.
6. After cooling to RT, add 100 µl isopropyl alcohol, then overturn to mix thoroughly, maybe appear the flocculated precipitate.
7. Transfer the last step solution and the flocculated precipitate into a Spin-column AC (place the Spin-column AC to Collection Tube), then centrifuge at 10,000 rpm for 30 min, discard flow-through.
It is important for above step to mix thoroughly, or decrease DNA yields. If the mixture is too sticky, please vortex 15 seconds.
8. Add 500µl buffer IR, centrifuge at 12,000 rpm for 30 sec, and discard flow-through.
9. Add 700µl buffer WB (**please check if ethanol added!**), centrifuge at 12,000 rpm for 30 sec and discard flow-through.
10. Add 500µl buffer WB, centrifuge at 12,000 rpm for 30 sec and discard flow-through.
11. Place Spin-column AC back to Collection Tube, centrifuge at 13,000 rpm for 2 min to remove all the ethanol in the column.
12. Take the Spin-column AC out, then put it into a clean tube, add 100µl buffer EB (incubated at 65-70°C water-bath), let it stand for 3-5 min in RT, centrifuge at 12,000 rpm for 1 min.
Add the flow-through back in the Spin-column AC, let it stand for 3-5 min at RT. Centrifuge at 12,000 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.
13. Keep DNA at 2-8°C. For long-term storage, please store it at -20°C

Troubleshooting

Problem	Possible Reason	Solutions
Low DNA yield	Proteinase K low or no activity	Please store aliquots under -20 °C avoiding multiple freeze-thaw.
	Lysis not adequate, or not mixed with isopropyl alcohol thoroughly.	Add Buffer CB and Proteinase K, then overturn to mix thoroughly Add isopropyl alcohol and then overturn to mix thoroughly then put in spin-column. If too sticky, vortex for 15 seconds.
	Some Gram-positive bacteria need special lysozyme.	Please read step 3, to understand the characters of extracting bacteria.
No Product	Ethanol not added to Buffer WB.	Add the ration ethanol before first use.
Low eluted DNA	Ethanol remains in Spin-column AC or Collection Tube bottom.	Ensure do step 10, or affect the elution efficiency.
	Use water or other solution to replace Buffer EB.	Please read step 11 carefully, just use Buffer EB.
A260 too high	Silicified membrane affects A260 value.	Centrifuge the DNA eluate at 13,000 rpm for 1 minute, and carefully take the supernatant for use.
DNA digestion inhibition	Silicified membrane inhibits digestion.	Centrifuge DNA eluate at 13,000 rpm for 1 minute; carefully take the supernatant for use.
	Ethanol remains in Spin-column AC or Collection Tube bottom.	Ensure do step 10, air dry at RT for a moment.