Note: for laboratory research use only.

Bacterial DNA Extraction Kit (Spin-column)

Cat. # DP2001 (50 preps)

DP2002 (100 preps)

ABigen corporation

www.abigen.com Tel:+86-010-13683383835 E-mail: info@abigen.com

Ⅰ. Kit Content, Storage and Stability

Content	Storage	50 preps (DP2001)	100 preps (DP2002)	200 preps (DP2003)
Buffer RB	RT	22 ml	44 ml	88 ml
Buffer CB	RT	11 ml	22 ml	44 ml
Buffer IR	RT	27 ml	50 ml	100 ml
Buffer WB	RT	15 ml	15ml×2	15ml×4
		Add the ration ethanol before use.		
Buffer EB	RT	15 ml	20 ml	40 ml
Isopropanol	RT	7 ml	14 ml	28 ml
Proteinase K (20mg/ml) (for type II)	-20℃	20mg freeze-dried powder	20mg×2 freeze-dried powder	20mg ×4 freeze-dried powder
Spin-column AC	RT	50	100	200
Collection Tube (2ml)	RT	50	100	200

All reagents are stable for 12 months when stored properly.

Notes:

1. **Please add 60ml ethanol to 15ml buffer WB before use, vortex adequately, and then mark on the block to avoid multi-adding!**
2. Buffer CB or IR may precipitate under low temperature; please incubate at 37℃ for a moment **until clear**, then cool down to RT for use.
3. **Proteinase K** is provided **in freeze-dried powder** for activity and transportation. Please **add 1ml sterile water to the tube after transient centrifugation upon arrival.** Because multiple freeze-thaw may affect enzyme activity, please store aliquots under -20℃.
4. Please keep all bottles' lids tightly after used to prevent reagents evaporating, oxidation and pH changing.

Ⅱ.Principle

The kit applies the unique binding buffer/ Proteinase K to rapidly lyse cells and inactivate cellular nucleases, and then DNA selectively absorbs on silicified membrane in high salt solution. Take a serial of elution-centrifugation steps to remove cellular metabolites and proteins etc, finally use low salt solution to elute the purified genomic DNA from silicified membrane.

Ⅲ.Features

1. The silicified membranes in the spin-column come from the world-famous company.
2. Poisonous phenol etc is not used.
3. Provide a very simple and fast way; single sample can be completed in 30 min.
4. Multi-elution can ensure high-purified DNA. The typical ratio of OD_{260}/OD_{280} is 1.7～1.9, and the average length is 30kb-50kb. Purified DNA can be applied for PCR，Southern-blot and digestions directly.

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. Please set water-bath at 70℃ before use.
3. For gram-positive bacteria, please prepare the lysozyme, or lysostaphin by yourself.
4. Buffer CB and IR contain the stimulating compound; please wear latex gloves to avoid skin, eyes and cloth to be contaminated. **If that, please use water or physiological saline washing.**

Ⅴ.Procedure

Please add 60ml ethanol to 15ml Buffer WB before first use!

1. Harvest cells (maximum 2×10^{9} cells) in a microcentrifuge tube by centrifuging at 10,000rpm 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 200μl buffer RB to suspend and wash cells. Centrifuge at 10,000rpm for 30sec, 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℃ 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℃ water-bath for 30-60 min. centrifuge at 10,000rpm 2min，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 (25mg/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℃ water-bath for 10 min.
6. After cooling to RT , add 100μl 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 10,000rpm 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 2min 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℃ 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℃. For long-term storage, please store it at -20℃.

Ⅵ.Troubleshooting

Problem	**Possible Reason Solutions**	
Low DNA yield	Proteinase K low or no activity	Please store aliquots under -20℃,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 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.
A_{260}too high	Silicified membrane affects A_{260} 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.

Telephone:
General (Ordering): +86-10- 13683383835
Email:
Technical Service：info@abigen.com
Ordering Information: info@abigen.com

ABigen corporation