Note: for laboratory research use only

Bacterial DNA Extraction Kit (Spin-column)

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

Cat. #: DP2001 (50 preps)

Signalway Biotechnology

Ⅰ. Kit Content, Storage and Stability

Content	Storage	50 preps (DP2001)
Buffer RB	RT	22 ml
Buffer CB	RT	11 ml
Buffer IR	RT	27 ml
Buffer WB	RT	15 ml
		Add 60 ml ethanol before use
Buffer EB	RT	15 ml
Isopropanol	RT	7 ml
Proteinase K (20 mg/ml) (for type II)	-20℃	20 mg (freeze-dried powder)
Spin-column AC	RT	50
Collection Tube (2ml)	RT	50

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

Reminder:

1 · ***Please add 60 ml ethanol to buffer WB before use; mix adequately and then check the box on the label showing ethanol was added!***

2 · Buffer CB or IR may precipitate under low temperature. Incubate the buffer at 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.

3 · **Proteinase K** is provided **in freeze-dried powder** for activity and transportation. **Add 1ml sterile water to dissolve.** Since multiple freeze-thaws may affect enzyme activity, store aliquots under -20℃.

4 · Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

Ⅱ.Principle:

The kit uses the unique binding buffer/proteinase K to rapidly lyse cells and inactivate cellular nucleases. DNA selectively adsorbs on silica membrane in high salt solution. A serial of elution-centrifugation steps will remove cellular metabolites and proteins etc. Low salt solution is used to elute the purified genomic DNA from silicified membrane.

Ⅲ.Features:

1・The kit does not contain poisonous phenol.

2・No need for ethanol precipitation.

3・Rapid and convenient. DNA can be extracted in 30 minutes

4・Multiple washing-elutions yield DNA in high purity, which can be applied to all kinds of molecular biology experiments such as PCR, Southern-blot, restriction enzyme digestion, and mammalian cell transfection.

Ⅳ.Notes

Read this section before your experiment.

1. All centrifugation steps can be performed at room temperature.
2. Buffers CB and IR contain a corrosive compound; please wear latex gloves to avoid contact with skin, eyes, and clothes**. If contact occurs, wash with water or physiological saline.**
3. Agarose gel electrophoresis or UV－spectrometer can be used for detecting the concentration and purity of DNA.
4. There is no EDTA in Buffer EB, which should not affect down-stream reactions. If interference occurs, use sterile water (pH >7.5) to elute and store DNA at -20℃. Low pH will decrease the elution efficiency. For long-term storage, elute DNA 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.

5. **For gram-positive bacteria, prepare the lysozyme or lysostaphin by users.**

Ⅴ.Procedure

Before Starting

- ***Dilute Buffer WB with 60 ml absolute ethanol, vortex adequately, and check the box on the bottle!***

1． Harvest cells (maximum 2×10^9 cells) by centrifugation at 10,000 rpm for 30 seconds. Collect bacterial pellet and discard the supernatant.

Initial sample amount depends on microbeing species and growth density. The maximum absorption of spin-column membrane is up to 25 μg genomic DNA. Avoid using too many cells that will decrease DNA yield.

2． Re-suspend the bacterial pellet by adding 250 μl Buffer RB. Then centrifuge at 10,000 rpm for 30 seconds, discard supernatant, then re-suspend pellet in 200 μl Buffer RB.

3． **For gram-negative bacteria (alternative)**：Re-suspend by adding 5 μl lysozyme (10 mg/ml in 10 mM Tris-HCl, pH 8.0), mix thoroughly, and incubate at 37℃ water-bath for 15 minutes.

For gram-positive bacteria：Re-suspend by adding 50-100 μl lysozyme (10 mg/ml in 10 mM Tris-HCl, pH 8.0), mix thoroughly，and incubate at 37℃ water-bath for 30-60 minutes. Centrifuge at 10,000 rpm for 2 minutes，discard supernatant, and then re-suspend 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. However, some bacteria, such as *Staphylococcus*, need add 25μl lysozyme (10 mg/ml) and 25 μl lysostaphin (10mg/ml) to disrupt cells.

4． **(Optional):** add 20 μl RNase A (25 mg/ml) solution, vortex to mix thoroughly, and stand at RT for 5-10 minutes to remove RNA.

5． Add 200 μl buffer CB and mix thoroughly. Add 20 μl Proteinase K (20 mg/ml), mix thoroughly，and incubate at 70℃ water-bath for 10 minutes.

6． After cooling to RT, add 100 μl isopropanol，and overturn to mix thoroughly (flocculated precipitate may appear).

For the steps above, “mixing thoroughly” is important for a higher yield. If the mixture is too sticky, please vortex 15 seconds.

7． Transfer the step 6 solution and the flocculated precipitate into a Spin-column AC (place the Spin-column AC to a Collection Tube), then centrifuge at 10,000 rpm for 30 min, discard flow-through**.**

8． Add 500μl buffer IR，centrifuge at 12,000 rpm for 30 seconds, and discard flow-through.

9． Add 700 μl buffer WB (**check if ethanol added**) ，centrifuge at 12,000 rpm for 30 seconds, and discard flow-through.

10. Add 500 μl buffer WB，centrifuge at 12,000 rpm for 30 seconds, and discard flow-through.

11. Place Spin-column AC back to a Collection Tube，centrifuge at 13,000 rpm for 2 minutes to remove all ethanol in the column.

12. Transfer the Spin-column AC to a clean tube, add 100 μl buffer EB (incubated at 65-70℃ water-bath), stand for 3-5 minutes at room temperature (RT), and centrifuge at 12,000 rpm for 1 min. Add the flow-through back in the Spin-column AC, stand for 3-5 minutes at RT, and centrifuge at 12,000 rpm for 1 minute.

Reduce 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.

13. Store DNA at -20℃ or apply for down-stream reaction.

Ⅶ. Troubleshooting

Problem	**Possible Reason**	**Solutions**
Low DNA yield	Low or no activity of proteinase K	Please store aliquots under -20℃, avoid multiple freeze-thaws.
	Lysis is not adequate. Mixing with isopropyl alcohol is not thoroughly.	Add Buffer CB and proteinase K, then **overturn to mix thoroughly.** Add isopropyl alcohol and **overturn to mix thoroughly**. 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 ethanol before use.**
Low eluted DNA	Ethanol remains in Spin-column AC or Collection Tube bottom.	Ensure to do step 10.
	Use water or other solution to replace Buffer EB.	Use Buffer EB.
A_{260} value is too high	Silicified membrane affects A_{260} value.	Centrifuge the DNA elutes at 13,000 rpm for 1 minute and carefully take the supernatant for use.
DNA digestion inhibition	Silica membrane inhibits digestion.	Centrifuge DNA elute at 13,000 rpm for 1 minute and carefully take the supernatant for use.

	Ethanol remains in Spin-column AC or Collection Tube bottom.	Ensure to do step 10, air dry at RT for a moment.