

Bacterial DNA Extraction Kit

(Solution type)

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

For laboratory research use only

DP1301 **50 preps**

Kit Content, Storage, and Stability

Content	Storage	50 preps
Nuclear Lysis Buffer	RT	33 ml
Protein Precipitation Buffer	RT	11 ml
DNA Dissolving Buffer	RT	10 ml
RNase A (10 mg/ml)	-20°C	100 ul

All reagents are stable for 12 months when stored properly.

Notes

1. Nuclear Lysis Buffer may form precipitation due to low storage temperatures. If necessary, dissolve the precipitation by 37°C water-bath and then cool to room temperature before use.
2. Protein Precipitation Buffer may form precipitation. If necessary, dissolve the precipitation by 37°C water-bath, or harvest upper-liquid for use.
3. Please ensure the bottles of buffer tightly capped when not in use, preventing reagents evaporating, oxidation and pH change.

Principle

This kit is applied to fast extract genomic DNA from bacteria. Nuclear lysis buffer destroys the cell wall and genomic DNA is released. RNA is removed by RNase. Protein precipitation solution precipitates and removes proteins selectively. Purified genome DNA is precipitated by isopropanol, and then DNA dissolved in DNA dissolving solution.

Features

1. Poisonous phenol etc not used.
2. Procedure is simple and fast, single sample can be completed in 60 min.
3. Stable result and high yield, high purity, the value of OD260/OD280 achieving 1.7-1.9. The Length of the genomic DNA is 50kb-150kb, which can be applied to PCR, Southern-blot and digestions directly.

Notes

Please read this section before your experiment.

1. All the centrifugation steps can be performed at room temperature.
2. 70% ethanol, 0.5M EDTA, lysozyme and water bath must be provided by customer
3. This kit is solution type, which can be proportionally amplified or narrowed to samples.

Procedure

1. Collect 1ml overnight culture and centrifuge at 9,000 rpm for 30 sec.
2. Discard the supernatant. For gram-positive bacteria, process step 3; for gram-negative bacteria, process step 6.
3. Add 480 µl mM EDTA to resuspend the pellet.
4. Add 120 µl lysozyme (10mg/ml) and mix thoroughly.

Lysozyme has a good lysis action on most of gram-positive bacteria, e.g. *Bacillus subtilis*, *Micrococcus luteus*, *Arthrobacter luteus*, *Nocardia otitidiscaviarum*, *Rhodococcus rhodochrous* and *Brevibacterium albidium*. But for some *Staphylococcus*, please add 60µl lysozyme and 60µl lysostaphin to ensure complete lysis.

5. Incubate at 37°C for 30-60 min. Centrifuge at 12.000 rpm for 2 min. Discard the supernatant.
6. Add 600 µl Nuclear Lysis Buffer and pipette gently.
7. Incubation at 80°C for 5 min and then cool to room temperature.
8. Add 1.8µl RNase A 10mg/ml to a final concentration of 30 µg/ml. Mix thoroughly and incubate for 15 min at 37°C to remove residues of RNA and then cool to room temperature.
9. Add 200 µl Protein Precipitation Buffer and vortex for 20-25 sec, some small protein masses appear. Incubate on ice bath for 5 min.
10. Centrifuge at 13,000 rpm for 5 min. White protein precipitation at the bottom of tube or in the surface of liquid will appear.
11. Carefully transfer supernatant to a new 1.5 ml centrifuge tube.
12. Add the equal volume isopropanol (about 600 µl) and gently overturn 30 times till some white filamentous DNA precipitation appears.
13. Centrifuge at 12,000 rpm for 1 min or vertically place centrifuge tube and let the precipitation down to the bottom, remove supernatant as much as possible keep the bottom white DNA precipitation.
14. Add 1 ml 70% ethanol and mix. Centrifuge at 13,000 rpm for 1 min, discard the supernatant.
15. Add 0.5 ml 70% ethanol, rinse DNA precipitation by reversal motion, centrifuge at 12,000 rpm for 1 min, discard supernatant thoroughly by pipetting and absorbent paper and air dry for several minutes.
The DNA will be difficult to dissolve because of excessive air dry. Don't leave too much ethanol, as it could inhibit the downstream endonuclease reaction.
16. Add 100 µl DNA (or adjust by concentration) dissolving buffer to dissolve DNA precipitation. Mix by tap the tube wall and incubate for 30-60 min at 65°C (no more than 1 h), or place stay over at room temperature or 4°C.
17. DNA could store at 2-8°C, or store at -20°C for long term storage.

Troubleshooting

Problem	Possible Reason	Solutions
Low DNA yield	Lysis buffer improperly use	Please take proper sample starting amount
	Some gram-positive bacteria need special enzyme for cell lysis.	Refer to step 4.
	DNA precipitation lost when washed.	Discard the supernatant after the ethanol washing carefully.
A260/A280>1.9	RNA contamination	Increase the amount of RNase A or prolonge the digestion time to 1h.
	DNA fragmentation.	Perform gently
No protein precipitation appear	The mixture not cool to room temperature before add protein precipitation.	Cool to room temperature or incubate for 5 min on ice, then add protein precipitation.
	No thoroughly mix protein precipitation solutions and cracking mixture.	Vortex for 25 sec, incubate on ice bath for 5min before centrifugation.
A260/A280 <1.6	Silicified membrane affects A260 value.	Refer to "No protein precipitation appear " and step 11
	Dilute DNA with water when measure the value of A260/A280	Use TE buffer instead of water, keep pH value >8.0.