11. Add 50 µl Elution Buffer, ddH_2O or TE Buffer to the Spin column and incubate for 1 min at room temperature. Avoid touching the membrane with the pipette tip.
    The volume of elution buffer added can be adjusted according to needs.
12. Centrifuge for 1 min at 12,000 x g. Discard the column.
13. The purified plasmid DNA can be used immediately for all kinds of downstream molecular biological experiments. Otherwise, store the purified plasmid DNA at -20°C.

Troubleshooting

1. **No Plasmid DNA purified**
   - If the plasmid DNA is not found in elution buffer, check whether the ethanol has been added to Wash Buffer according to the volume specified on bottle label.
2. **Low plasmid DNA yields**
   - Bacterial cultural too old. Inoculate a new batch of antibiotic-containing medium with a freshly isolated bacterial colony from an overnight plate. Incubate the cells for 12-16 hrs at 37°C.
   - Incomplete lysis of bacterial cells. The bacteria cell pellets should be resuspended completely in the resususpension buffer. No cell clumps should be visible after resuspension.
   - Incubate the Elution Buffer at 30~60°C prior to use to increase the yield.
3. **DNA yields low in spectrophotometric readings**
   - If the ratio of OD_{260} / OD_{230} is low, wash the spin column again.
   - If the ratio of $OD_{260-320}$ / $OD_{280-320}$ is low, there might be protein contamination. After adding Neutralization Buffer, centrifuge the buffer at an appropriate rotation speed. Avoid the precipitation while taking out the supernatant.
   - If the ratio of $OD_{260-320}$ / $OD_{280-320}$ is high, add Rnase A to Resuspension Buffer (100 mg/mL).
4. **Electrophoresis problem**
   - If there is genomic DNA in the result. Invert the tube gently instead of vortexing (steps 4 and 5).
   - If there is RNA in the result, add Rnase A to Resuspension Buffer (100 mg/mL).

RESEARCH INSTRUMENTS
Enabling Next-Generation Science

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PRODUCT INFORMATION

PureNA™ Biospin Plasmid Miniprep Kit
Cat# KN01-50, KN01-250

Kit Components

Cat#	KN01-50	KN01-250
Components	**50 preps**	**250 preps**
Resuspension Buffer	12.5 mL	62.5 mL
Lysis Buffer	12.5 mL	62.5 mL
Neutralization Buffer	17.5 mL	87.5 mL
Wash Buffer	30 mL (add 45 mL absolute ethanol before use)	75 mL (add 112.5 mL absolute ethanol before use)
Elution Buffer	10 mL	50 mL
RNase solution	50 µL	250 µL
Collection tubes (2 mL)	50	250

Storage and Stability

1. *PureNA*™ Biospin Plasmid Miniprep kit can be stored at room temperature (15~25°C) for up to 18 months except RNase solution. RNase solution should be stored at 2~8°C. After addition of RNase solution, Resuspension Buffer should be stored at 2~8°C.
2. The kit can be transported at room temperature.

Description

The kit provides a fast, simple and cost-effective purification of plasmid DNA from bacterial cultures. Plasmid DNA can be purified from 1–5 mL of overnight cultures of *E. coli*. The plasmid DNA isolated by *PureNA*™ Biospin Plasmid Miniprep kit is ready for use in downstream applications such as restriction enzyme digestion, sequencing, PCR/Real-time PCR and other downstream experiments.

Technical Information

Method	Work time	Column volume	Column yield	Elution recovery	Plasmid DNA length	Culture volume
Spin column	25 mins for 24 samples	750 µl	20 µg DNA	≥99%	≤10 kB	1~5 mL high-copy plasmid

Apparatus and materials to be prepared by the user

- Sterile 1.5 mL microcentrifuge tubes
- Absolute ethanol (≥95%)
- Microcentrifuge capable of 14,000 × g
- Vortex mixer

IMPORTANT NOTES

1. Add the provided RNase solution into the Resuspension Buffer before use. Mix well and store at 2-8°C.
2. Add ethanol (volume specified on bottle label) to Wash Buffer and mix well.
3. If the Lysis Buffer and Neutralization Buffer form a precipitate, it can be dissolved by warming the solution at 37°C. Do not shake the mixture too vigorously.
4. Close the lid immediately after using Lysis Buffer to avoid acidification.

Centrifugation Protocol

1. Add 1-1.5 mL cultured bacteria to 1.5 mL micro centrifuge tube.
2. Harvest bacterial culture by centrifugation for 30 sec at 10,000 rpm (8,000 ~ 10,000 x g) in a microcentrifuge. Decant the supernatant and remove all remaining medium. If you have >1.5 mL (up to 5 mL) bacterial culture, centrifuge the bacterial culture 3-5 mins at 5000 x g instead.
3. Add 250 µl Resuspension Buffer and completely resuspend the cell pellet by vortexing or pipetting until no cell clumps remain.
4. Add 250 µl Lysis Buffer and mix thoroughly by inverting the tube 4-6 times. *Do not vortex to avoid shearing of genomic DNA. Do not incubate for more than 5 mins.*
5. Add 350 µl Neutralization Buffer and mix thoroughly by inverting the tube 4-6 times. *The solution should become cloudy and no precipitate should be visible.*
6. Centrifuge for 10 mins at 13,000 rpm (>14,000 x g) until a compact white pellet forms.
7. Transfer the supernatant to the supplied Spin column and centrifuge for 30-60 sec at 6,000 x g. Discard the flow-through and place the column back into the same collection tube.
8. Add 650 µl Wash Buffer to the Spin column and Centrifuge for 30-60 sec at 12,000 x g. Discard the flow-through.
9. Repeat step 8 once.
10. Centrifuge for an additional 1 min at 12,000 x g and transfer the Spin column to a new, sterile 1.5 mL micro centrifuge tube. This step is recommended to remove residual ethanol.