3. Add 250 µl **Lysis Solution** and mix gently by inverting the tube 4-6 times until the lysate mixture is thoroughly homogenous. Do not vortex.
   • Do not incubate for more than 5 min.

4. Add 350 µl **Neutralization Solution** and mix immediately and thoroughly by inverting the tube 4-6 times. Do not vortex.
   • The neutralized bacterial lysate is cloudy and viscous.

5. Centrifuge at 12,000 rpm for 10 min.

6. Transfer the supernatant to the supplied spin column.
   • Do not over 700 µl one time. Avoid transferring the white precipitate.

7. Centrifuge at 12,000 rpm for 1 min. Discard the flow-through.
   • Do not add bleach to the flow-through.

8. Add 500 µl **Wash Buffer PB** to the spin column. Centrifuge at 12,000 rpm for 1 min and discard the flow-through.
   • Wash Buffer PB must be diluted with isopropyl alcohol previously.

9. Add 500 µl **Wash Buffer W** to the spin column. Centrifuge at 12,000 rpm for 1 min and discard the flow-through.
   • Wash Buffer W must be diluted with ethanol (96-100%) previously.

10. Repeat step 9 again.

11. Centrifuge at 12,000 rpm for 3 min to remove residual wash solution. Discard the collection tube with flow-through.

12. Place the spin column into a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl **Elution Buffer** to the center of the column without touching the membrane. Incubate at room temperature for 2 min and centrifuge at 12,000 rpm for 2 min.
   • Prewarm Elution Buffer to 65˚C will increase the yield of DNA, especially the plasmids or cosmids > 20 kb.
   • A second elution step will recover residual DNA from the membrane and increase the overall yield by 10-20%.

13. Discard the column. **Store the purified plasmid DNA at -20˚C**.

DNA Quantitation

Perform DNA quantitation using UV absorbance at 260 nm.

DONGSHENG BIOTECH

High Purity Plasmid Miniprep Kit

(High Purity, Silica - membrane Spin Column)

N1011 50 preps

N1012 100 preps

N1013 200 preps

For Research Use Only

www.dongshengbio.com/en/

Kit Contents

Component	N1011	N1012	N1013
	50 preps	100 preps	200 preps
RNase A (10 mg/ml)	150 µl	300 µl	600 µl
Resuspension Solution	15 ml	30 ml	60 ml
Lysis Solution	15 ml	30 ml	60 ml
Neutralization Solution	20 ml	40 ml	80 ml
Wash Buffer PB	18 ml	36 ml	72 ml
Wash Buffer W	30 ml	30 ml × 2	40 ml × 3
Elution Buffer (10 mM Tris-HCl, pH 8.5)	5 ml	10 ml	20 ml
Spin Columns	50 each	100 each	200 each

Description

High Purity Plasmid Miniprep Kit is designed for rapid and cost-effective small-scale extraction of high quality plasmid DNA from transformed *E.coli* cultures. The kit utilizes silica-based membrane technology in the form of a convenient spin column. Each spin column can recover up to 40 µg plasmid DNA. The kit can be successfully used for efficient purification of any size plasmid DNA and cosmid DNA. The actual plasmid yield and optimal culture volume depend on the plasmid copy number and medium used for cultivation.

The purification theory: silica particles bound DNA selectively at high salt concentration and low pH, while protein and other impurities are removed. The pure DNA is eluted with low salt buffer or water. This method requires few manipulations, and is both faster and easier to perform than other organic-based extraction methods.

Downstream Applications

Purified DNA is free of impurities and enzyme inhibitors, and have an $A_{260/280}$=1.7-1.9, is suitable for applications such as:

PCR/qPCR/Restriction enzyme digestion/Sequencing/*In vitro* transcription

Features

- **Fast** - procedure takes only 30 min
- **High efficiency** - 10-40 µg of plasmid DNA from one prep
- **High purity** - purified DNA without enzyme inhibitors, RNA or proteins, ready for downstream applications

Storage

Store RNase A at -20˚C, other reagents at room temperature for up to 1 year.

The Resuspension Solution with RNase A is stable for 6 months at 4 ˚C. Any precipitate in the Lysis Solution can be dissolved by incubating at 37˚C before use.

Important Notes

• Add all provided RNase A solution to the Resuspension Solution and mix well.

• Prior to the initial use of the kit, dilute the Wash Buffer PB, Wash Buffer W with isopropyl alcohol or ethanol (96-100%):

Solution	N1011 (50 preps)	N1012 (100 preps)	N1013 (200 preps)
Wash Buffer PB	18 ml	36 ml	72 ml
Isopropyl Alcohol	12 ml	24 ml	48 ml
Total Volume	30 ml	60 ml	120 ml

Solution	N1011 (50 preps)	N1012 (100 preps)	N1013 (200 preps)
Wash Buffer W	30 ml	30 ml × 2	40 ml × 3
Ethanol	45 ml	45 ml × 2	60 ml × 3
Total Volume	75 ml	75 ml × 2	100 ml × 3

Mix well, mark the labels on the bottle that isopropyl alcohol or ethanol is added.

• Ensure that no DNases are introduced into the sterile solutions of the kit.

• Make sure there is no precipitates in Lysis Solution. If any precipitate is visible, warming the solution at 37˚C for 3-5 min to dissolve the precipitate, and cooling to 25˚C before use.

• Wear disposable gloves when handling Lysis Solution, Neutralization Solution and Wash Buffer PB as they contain guanidine hydrochloride or other denaturing agent.

• All purification steps should be carried out at room temperature.

• All centrifugations should be carried out by a table-top microcentrifuge at>12,000 g (10,000-14,000 rpm, depending on the rotor type).

Preparation Work - Growth of Bacterial Cultures

Pick a single colony from a fresh selective plate to inoculate in LB medium with appropriate antibiotic. Incubate for 12-16 hours at 37˚C while shaking at 200-250 rpm. The bacterial culture should have a cell density of approximately 10^9 cells/ml or an absorbance of 1-1.5 at 600 nm (A_{600}).

Protocol

1. Harvest 1-5 ml overnight culture (1-2×10^9 *E. coli*). Centrifuge at 12,000 rpm for 1 min. Remove the medium thoroughly.
   • Do not overload: high-copy plasmids≦5 ml, low-copy plasmids≦10 ml.

2. Resuspend the cells in 250 µl **Resuspension Solution**. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
   • Ensure RNase A has been added to the Resuspension Solution.