High Purity Plasmid Miniprep Kit

For research use only

Content	DN1011	DN1012	DN1013
RNase A	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 DPB	30 ml	60 ml	120 ml
Wash Buffer DW	30 ml	60 ml	120 ml
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 preparation of high quality plasmid DNA from recombinant E.coli cultures. The kit utilizes an exclusive silica-based membrane technology in the form of a convenient
spin column. Each spin column can recover up to 40 µg of plasmid DNA. The kit can be successfully used for efficient purification of any size plasmids and cosmids. The actual plasmid yield and optimal culture volume depend on the plasmid copy number and medium used for cultivation.

Applications

Purified DNA is free from contaminants and enzyme inhibitors, and typically has A260/A280 ratios between 1.7 and 1.9, and is suitable for applications such as:

- **Restriction digestion**
- **PCR**
- **DNA Sequencing**
- **in vitro transcription**

Feature.

- **Fast - procedure takes only 30 min.**
- **Efficient – can get 10-40µg high copy plasmid DNA in one prep.**
- **High purity - Purified DNA is ready for downstream application such as PCR, restriction digestion.**

Store

Store RNase A at -20°C, other reagents can be store at room temperature for up to 1 year. Any precipitate forms in the buffers during storage, it should be redissolved by incubating the buffers at 37°C before use. After addition of RNase A, the Resuspension Solution can be used for 6 months when stored at 4°C.

Note

- **Add the provided RNase A solution to the Resuspension Solution and mix.**
- **Prior to the initial use of the kit, dilute the Wash Buffer W with ethanol (96-100%):**

	DN1011 (50 P)	DN1012 (100 P)	DN1013 (200 P)
Wash Buffer(DW)	30 ml	30 ml x2	30 ml x3
Ethanol	45 ml	45 ml x2	45 ml x3
Total Volume	75 ml	75 ml x2	75 ml x3

1. After the ethanol has been added, mark the check box on the bottle to indicate the completed step.

2. • Check the Lysis Solution and the Neutralization Solution for salt precipitation before each use. Redissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use. Do not shake the Lysis Solution too vigorously.

- **Both the Lysis Solution and the Neutralization Solution contain irritants. Wear gloves when handling these solutions.**
- **All purification steps should be carried out at room temperature.**

• All centrifugations should be carried out in a table-top microcentrifuge at>12000 g (10,000-14,000 rpm, depending on the rotor type)

Growth of Bacterial Cultures

1. Pick a single colony from a freshly streaked selective plate to inoculate 1-5 ml of LB medium supplemented with the appropriate selection antibiotic. Incubate for

12-16 hours at 37°C while shaking at 200-250 rpm. Use a tube or flask with a volume of at least 4 times the culture volume.

2. Harvest the bacterial culture by centrifugation at 8000 rpm (6800 x g) in a microcentrifuge for 2 min at room temperature. Decant the supernatant and remove all remaining medium.

Do not overload the column:

For high-copy-number plasmids (see Table 1), do not process more than 5 ml of bacterial culture. If more than 5 ml of such a culture are processed, the GeneJET™ spin column capacity (40 µg of dsDNA) will be exceeded and no increase in plasmid yield will be obtained.For low-copy-number plasmids (see Table 1), it may be necessary to process larger volumes of bacterial culture (up to 10 ml) to recover a sufficient quantity of DNA.

Table 1 Copy numbers of various vectors

High-copy (300-700 copies per cell)	Low-copy (10-50 copies per cell)	Very low-copy (Up to 5 copies per cell)
pUC vectors	pBR322 and derivatives	pSC101 and derivatives
pBluescript vectors	pACYC and derivatives	
pGEM vectors		
pTZ vectors		
pJET vectors		

Protocol

1. Pellet 1–5 ml of an overnight culture (1–2 x 109 E. coli in LB medium). Thoroughly remove all medium from the cell pellet.

2. Resuspened the pelleted cells in 250 µl of the Resuspension Solution. Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

Note. Ensure RNase A has been added to the Resuspension Solution.

3. Add 250 µl of the Lysis Solution and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear.

Note. Do not vortex to avoid shearing of chromosomal DNA. Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.

4. Add 350 µl of the Neutralization Solution and mix immediately and thoroughly by inverting the tube 4-6 times.

Note. It is important to mix thoroughly and gently after the addition of the Neutralization Solution to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate is cloudy and viscous.

5. Centrifuge for 10 min at 14,000 × g (12,000rpm) to pellet cell debris and chromosomal DNA.

6. Transfer the supernatant to the supplied spin column by decanting. Avoid disturbing or transferring the white precipitate.

7. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.

Note. Do not add bleach to the flow-through.

8. Add 500 µl of the Wash Buffer DPB to the spin column. Centrifuge for 30-60 seconds at 14,000 × g (12,000rpm) and discard the flow-through. Place the column back into the same collection tube.

9. Add 500 µl of the Wash BufferD W to the spin column. Centrifuge for 30-60 seconds at 14,000 × g (12,000rpm) and discard the flow-through. Place the column back into the same collection tube.

Note Wash Buffer DW must previously diluted with 100% ethanol.

10. Repeat step 9 again.

11. Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.

12. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl Elution Buffer DTE (prewarm to 60) directly to the center of the column without touching the membrane. Incubate for 2 min at room temperature and centrifuge for 2 min at 14,000 × g (12,000rpm).

Note. An additional elution step (optional) with Elution Buffer or water will recover residual DNA from the membrane and increase the overall yield by 10-20%. For elution of plasmids or cosmids >20 kb, prewarm Elution Buffer to 60°C before applying to silica membrane.

13. Discard the column and store the purified plasmid DNA at -20°C.

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