# GeneJET™ Plasmid Miniprep Kit

# (With some modification)

## DESCRIPTION

The GeneJET™ 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 GeneJET™ spin column can recover up to 20 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.

## PRINCIPLE

Pelleted bacterial cells are resuspended and subjected to SDS/alkaline lysis (1) to liberate the plasmid DNA. The resulting lysate is neutralized to create appropriate conditions for binding of plasmid DNA on the silica membrane in the spin column (2). Cell debris and SDS precipitate are pelleted by centrifugation, and the supernatant containing the plasmid DNA is loaded onto the spin column membrane. The adsorbed DNA is washed to remove contaminants, and is then eluted with a small volume of the Elution Buffer (10 mM Tris-HCl, pH 8.5). The purified plasmid DNA is ready for immediate use in all molecular biology procedures such as conventional digestion with restriction enzymes, fast digestion with FastDigest® restriction enzymes, PCR, transformation and automated sequencing.

### Buffer Preparation

- Add the provided **RNase A** solution to the **Resuspension Solution** and mix. After addition of RNase A, the Resuspension Solution can be used for 6 months when stored at 4°C.
- Add ethanol (96-100%) to the **Wash Solution** prior to first use.
- 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.

### 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.

For **high-copy-number plasmids** (*300-700*), 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 (20 µg of dsDNA) will be exceeded and no increase in plasmid yield will be obtained.
For **low and medium copy-number plasmids** (*1-50*), it is necessary to process larger volumes of bacterial culture (up to **20 ml**) to recover a sufficient quantity of DNA.

## PURIFICATION PROTOCOL

**Note**

• All purification steps should be carried out at **room temperature**.
• All centrifugations should be carried out in a table-top microcentrifuge at **>12000 x g** (10 000-14 000 rpm, depending on the rotor type).
Use 1-5 ml of *E. coli* culture in LB media for purification of **high-copy** plasmids. For **low-copy** plasmids use up to 20 ml of culture.

**Procedure**

1. Resuspend the pelleted cells in **250 µl of the Resuspension Solution (use 500 µl for low copy plasmids)**. Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing until no cell clumps remain.

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

2. Add **250 µl of the Lysis Solution (500 µl for low copy plasmids** ) 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.

3. Add **350 µl of the Neutralization Solution (700 µl for low copy plasmids)** 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.

4. Centrifuge for 10 min to pellet cell debris and chromosomal DNA.

5. Transfer the supernatant to the supplied GeneJET™ spin column. Avoid disturbing or transferring the white precipitate.

6. 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,

7. Add 500 µl of the Wash Solution (diluted with ethanol prior to first use) to the GeneJET™ spin column. Centrifuge for 1 min and discard the flow-through. Place the column back into the same collection tube.

8. Repeat the wash procedure (step 7) using 500 µl of the Wash Solution.
9. 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.
10. Transfer the GeneJET™ spin column into a fresh 1.5 ml microcentrifuge tube (not included). Add 50 µl of the Elution Buffer to the center of GeneJET™ spin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 min at room temperature and centrifuge for 2 min.

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 70°C before applying to silica membrane. (For downstream applications like PCR, restriction digestion, cloning it is recommended to use free water instead of elution buffer) for more concentrated DNA you can use 30 µl elution)

11 Discard the column and store the purified plasmid DNA at -20°C. (with some treatment the columns can be used again)

**TROUBLESHOOTING**

**A- Low yield of plasmid DNA**

**Bacterial culture too old**

Inoculate a fresh batch of antibiotic-containing growth medium with a freshly-isolated single bacterial colony from an overnight plate. Cultivate the cells for no more than 16 h at 37°C while shaking in LB media. Reduce the cultivation time to less than 12 h when using rich media like TB.

**Incomplete lysis of bacterial cells**

It is essential that the cell pellet is completely resuspended in the Resuspension Solution prior to lysis. No cell clumps should be visible before the addition of the Lysis Solution. Check the Lysis Solution for salt precipitation before each use. Redissolve any precipitate by warming the solution to 37°C, then mix well before use. Cell cultivation in LB media is recommended. Reduce culture volume when using a rich cultivation media like TB.

**Inefficient elution of DNA**

The Elution Buffer must be dispensed to the center of the membrane for efficient elution.

**Low copy plasmid**

Use larger volume of culture

## B- Contaminated DNA preparation

**Residual ethanol**

Ensure that step 9 of the protocol is performed you can repeat.

**RNA in the eluate**

Ensure that RNase A is added to the Resuspension Solution before the first use.

**Genomic DNA in the eluate**

To avoid shearing and liberation of genomic DNA, do not vortex or shake the cells during lysis and neutralization (steps 2 and 3), mix by gentle inversion of the tube.
Do not lyse the cells (step 2) for more than 5 min.
Do not cultivate cells longer than 16 h in LB media or 12 h in TB media.

**Additional band of denatured plasmid**

Denatured plasmid DNA migrates ahead of supercoiled DNA. It is not suitable for following enzymatic manipulations such as restriction digestion. To avoid denaturation, do not lyse the cells (step 2) for more than 5 min.

## References

1. Birnboim H.C., and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7, 1513 -1522.
2. Vogelstein, B. and Gillespie, D. (1979) Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76, 615-619.

**SAFETY INFORMATION**

**Lysis Solution**

**Xi** Irritant

**Risk phrases**

R36/38 Irritating to eyes and skin.

**Safety phrases**

S23 Do not breathe gas/fumes/vapour/spray.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S37 Wear suitable gloves.

S60 This material and its container must be disposed of as hazardous waste.

**Neutralization Solution**

**Xn** Harmful

Hazard-determining components of labeling:

**guanidinium chloride**

**Risk phrases**

R22 Harmful if swallowed.

R36/38 Irritating to eyes and skin.

**Safety phrases**

S23 Do not breathe gas/fumes/vapour/spray.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S36/37 Wear suitable protective clothing and gloves.

S60 This material and its container must be disposed of as hazardous waste.