# GeneJET™ Plasmid Miniprep Kit

**#K0501, #K0502, #K0503**

## CONTENTS





## COMPONENTS OF THE KIT

| GeneJET™ Plasmid Miniprep Kit | 25 preps<br>#K0501 | 50 preps<br>#K0502 | 250 preps<br>#K0503 |
|---|---|---|---|
| Resuspension Solution | 7ml | 15ml | 70ml |
| Lysis Solution | 7ml | 15ml | 70ml |
| Neutralization Solution* | 10ml | 20ml | 100ml |
| Wash Solution (concentrated) | 10ml | 20ml | 100ml |
| RNase A | 0.07ml | 0.15ml | 0.7ml |
| Elution Buffer | 2ml | 4ml | 30ml |
| GeneJET™ Spin Columns | 25 | 50 | 250 |
| Collection Tubes (2ml) | 25 | 50 | 250 |

* Wear gloves when handling Neutralization Solution. Do not add bleach to sample preparation waste.

## STORAGE AND STABILITY

GeneJET™ Plasmid Miniprep Kit can be stored for up to 12 months at room temperature (15-25°C). For longer storage, it is recommended to keep the kit at 4°C.

If 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.

## SAFETY INFORMATION

The following components of the GeneJET™ Plasmid Miniprep Kit contain hazardous contents. Wear gloves and goggles when working with those solutions.

Do not add bleach to sample preparation waste.

**Lysis Solution** contains sodium hydroxide, which is irritant.
Risk and safety phrases: R36/38, S 26-37/39-46.

**Neutralization Solution** contains guanidine hydrochloride, which is harmful and irritant.
Risk and safety phrases: R 22-36/38, S 23.

**RNase A Solution** contains ribonuclease, which is irritant.
Risk and safety phrases: R42/43, S 23-24-26-36/37

**Risk phrases**: R22: harmfull if swallowed, R36/38: irritating to eyes and skin, R42/43: may cause sensitization by inhalation and skin contact.

**Safety phrases**: S23: do not breathe spray, S24: avoid contact with skin, S26: in case of contact with eyes, rinse immediately with plenty of water and seek medical advice, S36/37: wear suitable protecting clothing and gloves, S37/39: wear suitable protecting clothing, gloves and eye/face protection, S46: if swallowed seek medical advice immediately and show the label.

## 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 20µg of plasmid DNA. 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.

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 endonucleases, PCR, transformation and automated sequencing.

## IMPORTANT NOTES

### 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:

| | **25 preps** #K0501 | **50 preps** #K0502 | **250 preps** #K0503 |
|---|---|---|---|
| Wash Solution (concentrated) | 10ml | 20ml | 100ml |
| Ethanol 96% | 17ml | 35ml | 170ml |
| Total Volume | 27ml | 55ml | 270ml |

- 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. *See* p. 2 for SAFETY INFORMATION.



### Growth of Bacterial Cultures

1. Pick a single colony from a freshly streaked selective plate to inoculate 1-5ml of LB medium supplemented with the appropriate selection antibiotic. Incubate for 12-16 hours at 37°C while shaking at 200-250rpm.
   Use a tube or flask with a volume of at least 4 times the culture volume.
2. Harvest the bacterial culture by centrifugation at 8000rpm (6800 x g) in a microcentrifuge for 2min at room temperature. Decant the supernatant and remove all remaining medium.

Do not overload the column:

For **high-copy-number plasmids** (*see* Table1), do not process more than **5ml** of bacterial culture. If more than 5ml of such a culture are processed, the GeneJET™spin column capacity (20µg of dsDNA) will be exceeded and no increase in plasmid yield will be obtained.

For **low-copy-number plasmids** (*see* Table1), it may be necessary to process larger volumes of bacterial culture (up to **10ml**) to recover a sufficient quantity of DNA.

**Table 1.** Copy numbers of various vectors

| **High-copy**<br>300-700 copies per cell | **Low-copy**<br>10-50 copies per cell | **Very low-copy**<br>Up to 5 copies per cell |
|---|---|---|
| pUC vectors<br>pBluescript vectors<br>pGEM vectors<br>pTZ vectors<br>pJET vectors | pBR322 and derivatives<br>pACYC and derivatives | pSC101 and derivatives |

## 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 000rpm, depending on the rotor type).
- Before starting see p.p.3-4 for IMPORTANT NOTES.

Use 1-5ml of *E. coli* culture in LB media for purification of **high-copy** plasmids.
For **low-copy** plasmids use up to 10ml of culture.

| Step | Procedure |
|---|---|
| **1** | Resuspend the pelleted cells in **250µl 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. |

| | |
|---|---|
| **2** | Add **250µl of the Lysis Solution** and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear.<br>**Note:** Do not vortex to avoid shearing of chromosomal DNA. Do not incubate for more than 5min to avoid denaturation of supercoiled plasmid DNA. |
| **3** | Add **350µl of the Neutralization Solution** and mix immediately and thoroughly by inverting the tube 4-6 times.<br>**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 5min to pellet cell debris and chromosomal DNA. |
| **5** | Transfer the supernatant to the supplied GeneJET™ spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate. |
| **6** | Centrifuge for 1min. Discard the flow-through and place the column back into the same collection tube.<br>**Note:** Do not add bleach to the flow-through, *see* p.3 for Safety Information. |
| **7** | Add **500µl of the Wash Solution** (diluted with ethanol prior to first use as described on p.3) to the GeneJET™ spin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube. |
| **8** | Repeat the wash procedure (step 7) using **500µl of the Wash Solution**. |
| **9** | Discard the flow-through and centrifuge for an additional 1min 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 1.5ml microcentrifuge tube (not included). Add **50µl 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 2min at room temperature and centrifuge for 2min.<br>**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%. |
| **11** | Discard the column and store the purified plasmid DNA at -20°C. |



## TROUBLESHOOTING

| Problem | Solution |
|---|---|
| **Low yield of plasmid DNA** | **Bacterial culture too old**<br>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 16h at 37°C while shaking in LB media. Reduce the cultivation time to less than 12h when using rich media like TB.<br><br>**Incomplete lysis of bacterial cells**<br>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.<br>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.<br>Cell cultivation in LB media is recommended. Reduce culture volume when using a rich cultivation media like TB.<br><br>**Inefficient elution of DNA**<br>The Elution Buffer must be dispensed to the center of the membrane for efficient elution. |
| **Contaminated DNA preparation** | **Residual ethanol**<br>Ensure that step 9 of the protocol is performed.<br><br>**RNA in the eluate**<br>Ensure that RNase A is added to the Resuspension Solution before the first use.<br><br>**Genomic DNA in the eluate**<br>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.<br>Do not lyse the cells (step 2) for more than 5min.<br>Do not cultivate cells longer than 16h in LB media or 12h in TB media.<br><br>**Additional band of denatured plasmid**<br>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 5min. |

## QUALITY CONTROL

The kit is tested for the isolation of pUC19 DNA from *E.coli* according to the protocol described above. The quality of isolated DNA is evaluated by spectrophotometric assay, agarose gel electrophoresis, digestion with FastDigest™ restriction endonucleases and automated fluorescent sequencing.

**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.


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