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PRODUCT INFORMATION

Thermo Scientific
GeneJET Plasmid Maxiprep Kit

#K0491, #K0492

www.thermoscientific.com/fermentas

Lot ___

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Plasmid Maxiprep Kit is qualified by isolating high copy number plasmid DNA from 250 ml of overnight *E. coli* culture (OD_{600} = 2) grown in LB medium following the protocol outlined in the manual. The kit passes QC requirements if the purified plasmid DNA has an $A_{260/280}$ ratio between 1.8 and 1.9 and a dominant band of supercoiled plasmid DNA is observed after agarose gel electrophoresis. The functional quality of the plasmid DNA is evaluated by digestion with restriction endonucleases.

Quality authorized by: Jurgita Zilinskiene

Rev.3.

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COMPONENTS OF THE KIT

GeneJET Plasmid Maxiprep Kit	**10 preps** #K0491	**25 preps** #K0492
Resuspension Solution	72 ml	180 ml
Lysis Solution	72 ml	180 ml
Neutralization Solution	72 ml	180 ml
Endotoxin Binding Reagent	9,6 ml	24 ml
Wash Solution I (concentrated)	75 ml	180 ml
Wash Solution II (concentrated)	75 ml	180 ml
RNase A Solution	3 x 1.0 ml	6 x 1.2 ml
Elution Buffer (10 mM Tris-HCl, pH 8.5)	18 ml	45 ml
GeneJET Maxi Purification Columns pre-assembled with collection tubes (50 ml)	10	25
Collection tubes (50 ml)	10	25

STORAGE AND STABILITY

Store Endotoxin Binding Reagent at 4°C. Other components of the kit should be stored at room temperature (15-25°C) and are stable for 1 year. For columns we recommend 4°C storage for periods greater than 1 year. The unopened vial of RNase A solution is stable at room temperature. Once the vial is opened, it should be stored at -20°C. Following the addition of RNase A Solution, the Resuspension Solution should be stored at 4°C and is stable for 6 months. **Any precipitate that forms in the buffers during storage can be redissolved by incubating briefly at 37°C, then cooling to room temperature before use.**

DESCRIPTION

The GeneJET™ Plasmid Maxiprep Kit is designed for large scale isolation of high quality plasmid DNA from recombinant *E.coli* cultures. The kit utilizes silica-based membrane technology in the form of convenient spin column. Each prep recovers up to 750 µg of high copy plasmid DNA that can be used in a wide variety of molecular biology procedures such as restriction endonuclease digestion, PCR, cloning, transformation, automated sequencing, *in vitro* transcription and transfection of robust cell lines.

PRINCIPLE

Pelleted bacterial cells are resuspended and subjected to SDS/alkaline lysis (1) to liberate plasmid DNA. The resulting lysate is neutralized to re-anneal plasmid DNA and precipitate proteins and chromosomal DNA. Cell debris and SDS precipitate are pelleted by centrifugation. The supernatant containing plasmid DNA is loaded onto the purification column. The high salt concentration of the lysate creates appropriate conditions for plasmid DNA binding to the silica membrane (2) in the spin column. The adsorbed DNA is washed to remove contaminants and eluted with the Elution Buffer.

IMPORTANT NOTES

Buffer preparation and handling

- Add the **RNase A Solution** provided in the kit to the **Resuspension Solution** and mix thoroughly. After the addition of RNase A, the Resuspension Solution is stable for 6 months when stored at 4°C.
- If the kit will be used infrequently, divide the Resuspension solution into an appropriate number of aliquots and supplement one aliquot with 40 µl of RNase A per 1 ml of Resuspension Solution to create a working solution. Store the remaining RNase A at -20°C. Sequential working solutions can be prepared by supplementing an aliquot of the Resuspension Solution with RNase A.
- Add the indicated volume of isopropanol to the **Wash Solution I** (concentrated) and ethanol (96-100%) to the **Wash Solution II** (concentrated) prior to the first use:

	10 preps (#K0491)		25 preps (#K0492)	
	Wash Solution I	**Wash Solution II**	**Wash Solution I**	**Wash Solution II**
Concentrated wash solution	75 ml	75 ml	180 ml	180 ml
Isopropanol	**25 ml**	**-**	**60 ml**	**-**
Ethanol (96-100%)	**-**	**125 ml**	**-**	**300 ml**
Total volume	100 ml	200 ml	240 ml	480 ml

- Check the Lysis Solution and the Neutralization Solution for salt precipitation before each use. Re-dissolve any precipitate by warming the solution to 37°C, then cool back down to 25°C. Do not vigorously shake the Lysis Solution.
- Wear gloves when handling the Lysis Solution and Wash Solution I bottles as these solutions contain irritants (see p.9 for SAFETY INFORMATION) and are harmful if they come in contact with skin, or if they are inhaled or swallowed.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and pipette tips
- Vortex
- Centrifuge capable of ~20,000 rpm (~48,000 x g) with rotor for appropriate centrifuge tubes
- Centrifuge with swinging bucket rotor capable of 2,000 – 5,000 x g
- Vacuum manifold (for vacuum protocol)
- Disposable gloves
- Ethanol (96-100%)
- Isopropanol

Bacterial strains

High quality plasmid DNA can be obtained from various *E.coli* strains including DH10B, DH5α, XL1-Blue, JM109, JM107, TOP10.

Culture media

The GeneJET Plasmid Maxiprep purification protocol outlined in this manual is optimized for use with bacterial cultures grown in Luria-Bertani (LB) medium to a cell density of approximately 2-3 (OD_{600} = 2-3). The use of rich growth media is not recommended.

Bacterial culture growth

- To prepare the bacterial culture, pick a single colony from a freshly streaked selective plate to inoculate 1-5 ml of LB medium supplemented with the appropriate antibiotic. Incubate for approximately 8 hours at 37°C while shaking at 200-250 rpm.
- Dilute the starter culture from 1:1,000 to 1:10,000 in LB medium and incubate for 12-16 hours (overnight) at 37°C while shaking at 200-250 rpm. The recommended bacterial culture optical density for plasmid DNA isolation is approximately 2-3 (OD_{600} = 2-3).
- Harvest cells by centrifugation at 5,000 x g for 10 min. Discard the supernatant.
- The bacterial pellet can be used immediately or stored at -20°C.

Culture volume

Generally, a 250 ml of overnight bacterial culture grown in LB medium is sufficient for good yield of high-copy and low-copy number plasmid DNA. However, it is important not to exceed recommended cell mass (culture volume x OD_{600}), because it may decrease quality of isolated DNA. The maximum culture volume to use can be determined using formula below:

Maximum culture volume (V), ml = 750/OD_{600}

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		

PROTOCOLS

A. Centrifugation protocol

Step	Procedure
1	Grow up to 250 ml of bacterial culture to an OD_{600} of 2-3 as outlined on p.4. **For best results calculate the maximum volume** of cell culture to use by referring to the "Culture volume" on p.4. Harvest bacteria cells by centrifugation for 10 min at 5,000 x g. Discard the supernatant.
2	Resuspend pelleted cells in **6 ml** of **Resuspension Solution**. The bacterial pellet can be resuspended by vortexing or pipetting up and down until no cell clumps remain. **Note.** Ensure that the RNase A Solution has been added to the Resuspension Solution as described on p.3.
3	Add **6 ml** of **Lysis Solution** and mix gently by inverting the tube 4-6 times until the solution becomes viscous and slightly clear. Incubate for **3 min** at room temperature. **Note.** Do not vortex to avoid shearing chromosomal DNA. Do not incubate for more than 3 min to avoid denaturation of supercoiled plasmid DNA.
4	Add **6 ml** of **Neutralization Solution** and mix immediately by inverting the tube 5-8 times.
5	Add **0.8 ml** of the **Endotoxin Binding Reagent** and mix immediately by inverting the tube 5-8 times. Incubate 5 min at room temperature. **Note.** After the addition of the Neutralization Solution and Endotoxin Binding Reagent it is important to mix the contents of the tube gently, but thoroughly, to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should appear cloudy and contain white precipitate.
6	Centrifuge for 20 min at 20,000 rpm (48,000 x g) to pellet cell debris and chromosomal DNA. (*see* p.6 to continue with vacuum protocol)
7	Transfer the supernatant to a **50** ml tube (not provided) by decanting or pipetting. Avoid disturbing or transferring the white precipitate. Add 1 volume of 96% ethanol. Mix immediately and vigorously by inverting the tube 5-6 times.
8	Transfer part of the sample (~20 ml) to the **column**. Do not overfill the column. Centrifuge for 3 min at 2,000 x g in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube.
9	Repeat step 8 to process any remaining lysate through the purification column.
10	Add **8 ml** of **Wash Solution I** (with isopropanol added as described on p.3) to the purification column. Centrifuge for 2 min at 3,000 x g in a swinging bucket rotor. Discard the flow-through and place the column back into the same collection tube.
11	Add **8 ml** of **Wash Solution II** (with ethanol added as described on p.3) to the purification column. Centrifuge for 2 min at 3,000 x g in a swinging bucket rotor. Discard the flow-through and place the column into the same collection tube.
12	Repeat the column wash with **Wash Solution II** (step 11).
13	Centrifuge for 5 min at 3,000 x g in a swinging bucket rotor to remove residual wash solution. Discard the collection tube containing the flow-through.

14	Transfer the column to a fresh 50 ml collection tube (provided). Add **1 ml** of **Elution Buffer** to the centre of the purification column membrane. Incubate for 2 min at room temperature and centrifuge for 5 min at 3,000 x g to elute plasmid DNA. **Note.** To increase the concentration of eluted DNA the volume of the Elution Buffer can be reduced to 0.7 ml. Be aware that lower volumes of Elution Buffer will decrease the overall yield of eluted DNA. To increase the overall DNA yield by 20-30% an additional elution step (optional) with Elution Buffer (0.5 ml) may be used.
15	Discard the purification column. Use the purified plasmid DNA in downstream applications or store at -20°C.

B. Vacuum Protocol

1-6	Perform cell collection, lysis and lysate clearing steps (step 1 to step 6) as outlined in the centrifugation protocol on p.5.
7	Transfer the supernatant to a **50 ml** tube (not provided) by decanting or pipetting. Avoid disturbing or transferring the white precipitate. Add 1 volume of 96% ethanol. Mix immediately by vigorously inverting the tube 5-6 times.
8	Prepare the vacuum manifold according to the supplier's instructions. Place the GeneJET Maxi Purification column(s) onto the manifold.
9	Transfer part of the sample (~20 ml) to the **column**. Be careful not to overfill the column. Apply vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.
10	Repeat step 9 to process the remaining lysate through the purification column.
11	Add **8 ml** of **Wash Solution I** (diluted with isopropanol as described on p.3) to the purification column. Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.
12	Add **8 ml** of **Wash Solution II** (diluted with ethanol as described on p.3) to the purification column. Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.
13	Repeat the column wash with **Wash Solution II** (step 12).
14	To dry the column, apply the vacuum for 10 min or transfer the GeneJET Maxi Purification Column into a fresh 50 ml collection tube (provided) and centrifuge for 5 min. at 3,000 x g in a swinging bucket rotor.
15	Transfer the Column to a fresh 50 ml collection tube (provided). Add **1 ml** of **Elution Buffer** to the centre of the column membrane. Incubate the column for 2 min at room temperature and centrifuge for 5 min at 3,000 x g in a swinging bucket rotor to elute plasmid DNA. **Note**. To increase the concentration of eluted DNA volume of the Elution Buffer can be reduced to 0.7 ml. Be aware that lower volumes of Elution Buffer will decrease the overall yield of eluted DNA. To increase the overall DNA yield by 20-30% an additional elution step (optional) with Elution Buffer (0.5 ml) may be used.
16	Discard the purification column. Use the purified plasmid DNA in downstream applications or store DNA at -20°C.

TROUBLESHOOTING

Problem	Possible cause and solution
Low plasmid DNA yield	**Incomplete bacterial cell lysis.** It is essential that the cell pellet is completely resuspended in the Resuspension Solution prior to lysis. Cell clumps should not be visible before the addition of 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 and cool to 25°C before use. Do not use more biomass than recommended. Refer to the "Culture volume" on p.4. **Isopropanol was not added to Wash Solution I.** Ensure that isopropanol **was added to Wash Solution I before the first use. Follow the instructions** to prepare Wash Solution I on p.3. **Ethanol was not added to Wash Solution II.** Ensure that ethanol was added to Wash Solution II before the first use. Follow the instructions to prepare Wash Solution II on p.3. **Purification column clogs during the purification procedure.** Reduce the volume of cell culture biomass processed per column. Avoid transferring pelleted cell debris to the purification column when loading the lysate to the column. **Suboptimal cell culture volume.** Calculate the maximum culture volume using the following formula: Maximum culture volume (V), ml = $750/OD_{600}$. **Old Bacterial culture.** Prepare a new starter culture by inoculating a freshly-isolated single bacterial colony in antibiotic-containing growth medium and grow bacteria as described in the "Bacterial Culture Growth" section on p.4.
Suboptimal $A_{260/280}$ ratio	**Purification of plasmid DNA is not efficient.** Reduce volume of cell culture. Follow recommendations described in "Culture volume", p.4.
Genomic DNA contamination	**Samples vigorously vortexed or shook during cell lysis or neutralization steps.** To avoid genomic DNA contamination, mix the solution (steps 3 and 4) by gently inverting the tubes 4-8 times. Do not allow the cell lysis step (step 3) to proceed for more than 3 min. Do not cultivate cells longer than 16 h in LB media. Residual genomic DNA can be removed from purified plasmid DNA using T7 DNA Polymerase (#EP0081).
RNA contamination	**RNase A Solution was not added to the Resuspension Solution before the first use.** Ensure that the RNase A Solution was added to the Resuspension Solution as described on p.3.
Purified prep contains additional plasmid forms	**Plasmid DNA denatured during cell lysis.** Denatured plasmid DNA migrates ahead of supercoiled DNA and is not suitable for enzymatic manipulations such as restriction digestion. To avoid denaturation, do not lyse the cells (step 3) for more than 3 min.

Inhibition of downstream enzymatic reactions	**Purified plasmid DNA contains ethanol.** Dry the purification column sufficiently prior to the DNA elution (step 13 of centrifugation protocol or step 14 of vacuum protocol).
No or low yields of *in vitro* transcription reaction products	**Purified plasmid DNA contains RNase A.** If the purified DNA is used for *in vitro* transcription, linearized plasmid DNA can be repurified using the GeneJET PCR Purification Kit (#K0701) or by phenol/chloroform extraction.

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.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/fermentas for Material Safety Data Sheet of the product.

SAFETY INFORMATION

Lysis Solution

C Corrosive

Hazard-determining components of labeling: **sodium hydroxide**

Risk phrases

R34 Causes burns.

Safety phrases

S20 When using do not eat or drink.
S23 Do not breathe gas/fumes/vapor/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S60 This material and its container must be disposed of as hazardous waste.

Neutralization solution

Xi Irritant

Hazard-determining components of labeling: **acetic acid**

Risk phrases:

R36/38 Irritating to eyes and skin.

Safety phrases:

23 Do not breathe gas/fumes/vapour/spray.
26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
37 Wear suitable gloves.
45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
60 This material and its container must be disposed of as hazardous waste.

Wash Solution I (concentrated)

Xn Harmful

Hazard-determining component of labeling: **guanidinium hydrochloride**

Risk phrases

R22 Harmful if swallowed.
R36/38 Irritating to eyes and skin.

Safety phrases

S23 Do not breathe gas/fumes/vapor/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.