PlasJia Mini Plasmid Extraction Kit

Isolation of Plasmid DNA (Based on silica technology)

- MiniPrep

For DNA Isolation from

Bacterial Cultured Cell

By ROJE

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ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable Molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

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Kit Content

Component	50 preps
SOB	12.5ml
PLB	12.5ml
NOT	17.5ml
PWB (Concentrated)	12.5ml
PEB	5ml
Prime RNase A	1.5mg
HiPure DR Column Column	50
Collection tube	100

Storage

PlasJia Mini Plasmid Extraction Kit is stable for 1 years when stored in a constant temperature 15 ~ 25°C. Prime RNase A is stable when stored at -15°C or -20°C.

Intended Use

The PlasJia Mini Plasmid Extraction Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease. The PlasJia Mini Plasmid Extraction Kit is designed for rapid and cost-effective small-scale preparation of high-quality plasmid DNA from recombinant bacterial 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 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.

Guarantee and Warranty

ROJETechnologies guarantee the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If products do not meet your satisfaction, please contact the technical support team for reasons other than misuse. If the problem is due to the manufacturing process, the ROJE team will replace the kit for you.

Notice to Purchaser

This product is only for experimental and not for commercial use of any kind. There is no right to resell the kit or any of its components. For information about our licensing or distributors, contact the ROJE business team.

Warning and Precautions

Due to chemical material usage that may be hazardous, always make sure to wear suitable lab coat, disposable gloves, and protective eyewear. Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.rojetechnologies.com.

* Buffers PLB and NOT contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling.

If precipitate forms in the buffers during storage, it should be redissolved by incubating the buffers at 37°C before use.

After addition of Prime RNase A, the Resuspension Solution can be used for 6 months when stored at 4°C.

Note: Close the bag with Spin Columns tightly after each use!

Quality control

PlasJia Mini Plasmid Extraction Kit is tested against predetermined experiments on a lot-to-lot basis according to ROJETechnologies ISO-certified quality management system, to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on web at www.rojetechnoloes.com.

Description

PlasJia Mini Plasmid Extraction Kit offers a simple, rapid and cost-effective method for purification of high copy and low copy plasmid DNA from bacterial cells. This kit is designed for the preparation of up 20 µg of high-purity plasmid DNA from 1 ~ 5 ml overnight E. *coli* culture in LB medium. Plasmid DNA purified with mini kit is immediately ready for use.

Procedure

In PlasJia Kit, pelleted bacterial cells are resuspended and subjected to SOB and PLB lysis buffers. The resulting lysate is neutralized with NOT Buffer to create appropriate conditions for the binding of plasmid DNA on the silica membrane in the spin column. 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 with PWB buffer to remove contaminants and is then eluted with a small volume of the PEB buffer. The purified plasmid DNA is ready for immediate use in all molecular biology procedures such as conventional digestion with restriction enzymes, PCR, transformation, and automated sequencing.

Equipment & Reagents to Be Supplied by User

- Ethanol (96-100%)
- Pipets and pipet tips
- 1.5 ml Microtube
- Vortex
- Centrifuge
- Micro centrifuge

Applications

The isolated DNA can be used in many downstream applications:

- Different kinds of PCRs, including Long-range PCR
- Sequencing
- Restriction digestion
- Southern blotting
- Cloning
- Ligation
- Transformation

Features

Specific features of Plasjia Kit are listed here in Table 1.

Table 1. PlasJia Kit features and specifications

Features	Specifications
Elution volume	30 ~ 100 µl
Technology	Silica technology
Main sample type	Bacterial cells cultured
Processing	Manual
Sample amount	1 ~ 5 ml bacterial cells cultured
Operation time per reaction	30 minutes
Typical yield	Up 20 µg
Average purity	A260/A280= 1.7-2.0
Size of DNA purified	Up to 40kb
Enzyme	Prime RNase A

Buffer Preparation

- Add Prime RNase A (1.5 mg) to SOB, mix and store at 4°C. After addition of Prime RNase A, the Resuspension Solution (SOB) can be used for 6 months when stored at 4°C.
- Add 12.5ml ethanol (96-100%) to PWB before use.

Table 1: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
PWB	12.5	12.5	25

Recommended Starting Material

Growth of Bacterial Cultures

- 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.
- Harvest the bacterial culture by centrifugation at 13,000 rpm in a microcentrifuge for 3 min at room temperature. Decant the supernatant and remove all remaining medium.

Do not overload the column

- For high-copy-number plasmids (see Table 2), do not process more than 5 mL of bacterial culture. If more than 5 ml of such a culture are processed, the spin column capacity (20 µg of dsDNA) will be exceeded and no increase in plasmid yield will be obtained.
- For low-copy-number plasmids (see Table 2), it may be necessary to process larger volumes of bacterial culture (up to 10 ml) to recover a sufficient quantity of DNA.

Table 2. 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

Protocol: Isolation of Plasmid DNA

Sample type:

- Bacteria Cultured Cell

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Do not forget to add the appropriate amount of ethanol (96%–100) to PWB as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- Check the Lysis Solution (PLB) and the Neutralization Solution (NOT) 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 (PLB) and the Neutralization Solution (NOT) contain irritants. Wear gloves when handling these solutions.

- Add Prime RNase A (1.5mg) to SOB, mix and store at 4°C. After addition of Prime RNase A, the Resuspension Solution (SOB) can be used for 6 months when stored at 4°C.

Process

1- Growth of bacterial cultures in tube or falcon and harvesting: Harvest the bacterial cells from 1 ~ 5ml recombinant E. *coli* culture by centrifugation of 13,000 rpm in a conventional, table-top micro-centrifuge for 3 min at room temperature.
2- Add 250 µl of SOB to the collected cells and completely re-suspend by vortexing or pipetting.
3- Add 250 µl of PLB and mix by inverting the tube 3~5 times by inverting, gently and incubate for 1min: Vortexing may cause shearing of genomic DNA. Do not vortex.
4- Add 350 µl of NOT and immediately mix by inverting the tube 3~5 times, gently: Genomic DNA and cell debris will be formed and insoluble complex. Do not vortex.
5- Centrifugation the tube at 13,000 rpm, 4 ~ 25°C for 10 min in micro-centrifuge: A compact white pellet will be appeared at the bottom of the tube.
6- Transfer the supernatant (cleared lysate) to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
7- Add 500 µl of Washing Buffer (PWB) to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
8- Dry the spin column by additional centrifugation at 13,000 rpm for 3 min to remove the residual ethanol in spin column.
9- Transfer the spin column to the new 1.5 ml micro-centrifuge tube (Not provided).
10- Add 30 ~ 100 µl of PEB or Nuclease free water to the spin column with micro-centrifuge tube, and let stand for at least 1 min.
11- Elute the plasmid DNA by centrifugation at 13,000 rpm for 1 min.
12- Discard the column and store the purified plasmid DNA at -20°C.

Troubleshooting

Here we try to cover as many problems as you may see in using this product, however scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

Problems	Possible Causes	Action
Low yield of plasmid DNA	Alkaline lysis was inefficient	If cells have grown to very high densities or a larger amount of cultured medium than recommended was used, the ratio of biomass to lysis reagent is shifted. This may result in poor lysis conditions, because the volumes of Buffers SOB, PLB and NOT are not sufficient for setting the plasmid DNA free efficiently. Reduce culture volume or increase volumes of Buffers SOB, PLB and NOT. Also, insufficient mixing of lysis reagents will result in reduced yield. Mix thoroughly after addition of Buffers SOB, PLB and NOT to achieve homogeneous suspensions.
	Insufficient lysis for low-copy plasmids	For low copy-plasmid preparations, doubling the volumes of buffers SOB, PLB and NOT may help to increase plasmid yield and quality.
	Lysate incorrectly prepared	Check Buffer PLB for SDS precipitation resulting from low storage temperatures and dissolve the SDS by warming. The bottle containing Buffer PLB

		should always be closed immediately after use.
	Column was overloaded	Check the culture volume and yield against the capacity of the Spin column, as detailed at the beginning of protocol. Reduce the culture volume accordingly, if a higher yield is desired. For very low-copy number plasmid requiring very large culture volumes.
	Buffer PH was too low	Ensure that the pH of the buffer used for redissolving is ≥8.0 because DNA does not dissolve well in acidic solutions.
The pellet is highly viscous	Resuspension volume too low	Increase resuspension volume if the solution above the pellet is highly viscous.
RNA in the elute	Prime RNase A digestion was insufficient	. Check culture volume against recommended volumes, and reduce if necessary. Check that the Prime RNase A provided with the kit has been used. If Buffer 1 is more than 6 months old, add more Prime RNase A.
Contamination	Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use new glass- and plasticware, and wear gloves.
Contaminated DNA preparation	Lysis time was too long	Ensure that lysis step (Buffer) does not exceed 5 min.
	Plasmid DNA is nicked/shared/degraded	DNA was poorly buffered. Redissolve DNA in TE buffer, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.

Ordering Information

category	Product name	Preps no.	Cat No.
DNA Technologies	**PlasJia Mini Kit**	50 Preps	DN983067
		100 Preps	DN013068

Symbols

Tables 3: symbols on the Kit Label

symbols	meaning	symbols	meaning
	Date of manufacture		manufacturer
	Expiration Date		Temperature limitation
-	-	LOT	Lot number
-	-	REF	Reference number

Technical Assistance

ROJETechnologies guarantees your complete satisfaction. ROJE technical support team composed of highly trained experienced scientists, who are able to troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select suitable product. Contact our technical support at any time by selecting one of these ways:

- Through our telephone and fax number; +982191070705.
- You can submit your question directly to ROJE Technical Support Team from our website (www.rojetechnologies. com) Or send your questions to this email address, technicalsupport@rojetechnologies.com.

Factory address

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