Fast DNA-spin™ Plasmid DNA Purification Kit

For the efficient isolation plasmid DNA

RUO *Research Use Only* REF *17013* Σ 200 15℃ 25℃

PRODUCT FEATURES

- Fast DNA-spin™ Plasmid DNA Purification Kit is designed for rapid purification of plasmids from bacterial cells.
- The fastest, simplest procedure for purifying the highest quality plasmid DNA.
- The high quality plasmid DNA can be used directly for the downstream application.
- Minimal nicks of plasmid DNA guarantees good results in plasmid DNA sequencing.

INTRODUCTION

The method that used in Fast DNA-spin™ Plasmid DNA Purification Kit is optimized from traditional alkaline lysis technology by which high quality plasmid DNA could be purified within 10 minutes. The new lysis buffer allows the adsorption of DNA onto silica membrane in the presence of high salt. The material that is used to make the silica membrane is unique, highly-efficient and highly-specified. This protocol is designed for purification of DNA from 1-4 ml overnight cultures of *E. coli*. Plasmid DNA prepared by Fast DNA-spin™ Plasmid DNA Purification Kit is suitable for a variety of routine applications including restriction enzyme digestion, sequencing, library screening, ligation and transformation, *in vitro* translation and transfection of robust cells.

KIT CONTENTS

Components	200 prep
Resuspension buffer[1] (Bottle 1)	60 ml
Lysis buffer[2] (Bottle 2)	60 ml
Neutralization buffer (Bottle 3)	80 ml
Washing Buffer[3] (Bottle 4)	50 ml
Elution Buffer (Bottle 5)	30 ml
Lysis Viewer	300 µl
RNase A[4] (10 mg/ml)	600 µl
Spin Column	200 ea
Collection Tube	200 ea
Storage Conditions : Room Temperature	

[1] Briefly spin the dissolved RNase A solution and add the RNase A solution to Resuspension Buffer. Before use, store Resuspension Buffer at 4℃ after adding RNase A solution.

[2] Check Lysis Buffer for SDS precipitation due to low storage temperature in which case it is necessary to dissolve the SDS by warming at 37℃.

[3] Before use, add 50 ml of absolute EtOH to the Washing buffer before use.

[4] RNase A (10 mg/ml) can be stored for one year at room temperature (15-25℃).

STORAGE CONDITION

The Fast DNA-spin™ Plasmid DNA Purification Kit can be stored at room temperature (15-25℃) for up to 12 months without showing any reduction in performance and quality. For longer storage, this kit can be stored at 2-8℃. If any precipitate forms in the buffers after storage at 2 - 8℃, it should be dissolved by warming the buffers at 37℃ before use. After addition of RNase A and Lysis Viewer, Resuspension Buffer is stable for 6 months at 2 - 8℃.

IMPORTANT NOTES

1. Add the provided RNase A and Lysis Viewer solutions to Resuspension Buffer before use, mix and store at 2 - 8℃.
2. Check Lysis Buffer and Neutralization Buffer before use for salt precipitation. Redissolve any precipitate by warming at 37℃.
3. Avoid direct contact of Lysis Buffer and Neutralization Buffer, immediately close the lid after use.
4. All centrifugation steps are carried out at 12,000rpm (~13,400 x g) in table-top micro-centrifuge at room temperature (15 - 25℃).
5. The obtained plasmid amount is influenced by bacteria culture density and plasmid copy number as well.
6. Lysis Viewer user guide : Lysis Viewer is an indicator which is harmless and used to make sure that the whole experimental process works well. Lysis Viewer should be mixed with Resuspension Buffer in the ratio of 1 : 200 and the color of the mixed solution should be clear red. Add the mixed solution to cell culture and the solution would turn turbid red. After that, add Lysis Buffer to the turbid solution, the solution would turn clear purple which means a complete lysis. Add Neutralization Buffer to the purple solution and it would turn clear yellow, which indicate that the neutralization reaction has been done.

BACTERIAL CULTURE and COLLECTION

1. Inoculate 1 - 10ml of LB medium containing the appropriate antibiotic either with a single colony of transformed bacteria or with 0.2ml ~ 1.0ml of a small-scale LB culture grown from a single colony.
2. Incubate the culture at 37℃ with shaking for 12 - 16 hours.
   - If you use ampicillin as an antibiotic for culture (OD_{600} 1.5 ~ 2.0), we recommend to increase your working ampicillin concentration up to 200 ~ 300µg/mL to sustain selective antibiotic pressure for obtaining higher plasmid yield.
   - Growth for more than 16h is not recommended since cells begin to lyse and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times volume of the culture.

PROTOCOL

1. Harvest 1 - 4ml bacterial cells in a microcentrifuge tube at 12,000rpm (~13,400 x g) in a conventional, table-top microcentrifuge for 1 min at room temperature (15 - 25℃), then remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.
2. Resuspend pelleted bacterial cells in 150µl Resuspension Buffer by pipetting or vortex (Ensure that RNase A and Lysis Viewer have been added to Resuspension Buffer).

   Note : Cell clumps indicate incomplete lysis, will result in lower yield and purity. Addition of Lysis Viewer will not have negative impact on following PCR, enzyme digestion and sequencing.
3. Add 150µl Lysis Buffer and mix gently by inverting the tube 6 - 8 times.

   Note : Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. If not clear, probably due to incomplete lysis, please reduce the cells.
4. Add 350µl Neutralization Buffer and mix immediately and quickly by inverting 12 - 20 times. The solution should become cloudy. Centrifuge for 2 min at 12,000rpm (~13,400 x g) in a table-top microcentrifuge.

   Note : To avoid localized precipitation, mix the solution quickly, immediately after addition of Neutralization Buffer. The solution should be centrifuged again if there is still a lot of white precipitate can be seen in the supernatant. Since Lysis Viewer is applied, after the addition and mix of Neutralization Buffer, the solution should turn clear yellow. If there is still some purple liquid can be seen in the tube, keep inverting the tube until the color of solution turns completely clear yellow.
5. Transfer the supernatant from step 4 to the Spin Column (put in a Collection Tube) by pipetting. Centrifuge for 30 s at 12,000rpm (~13,400 x g). Discard the flow-through and set the Spin Column back into the Collection Tube.
6. Wash the Spin Column by adding 300µl Washing Buffer (ensure the absolute ethanol has been added to Washing Buffer) and centrifuging for 30s at 12,000 rpm (~13,400 x g). Discard the flow-thorough, and put the Spin Column back into the Collection Tube.
7. Centrifuge for an additional 1 min at 12,000 rpm (~13,400 x g) to remove residual Washing Buffer.
8. Place the Spin Column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 - 100µl Elution Buffer to the center of the Spin Column, centrifuge for 30 s at 12,000rpm (~13,400 x g).

   Note : the volume of eluted buffer should not be less than 50µl, otherwise it may affect recovery efficiency. The pH value of eluted buffer will have a great effect on eluting.

NOTICE BEFORE USE

Fast DNA-spin™ Plasmid DNA Purification Kit is intended for research use only. And Fast DNA-spin™ Plasmid DNA Purification Kit is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. Please observe general laboratory precaution and utilize safety while using this kit.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

At iNtRON we take pride in the quality and availability of our technical support. iNtRON is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experience any difficulties regarding the **Fast DNA-spin™ Plasmid DNA Purification Kit**, please do not hesitate to contact us. iNtRON customers are a major source of information regarding advanced or specialized uses of our products. For technical assistance and more information please call iNtRON's local distributors.

TECHNICAL ADVICE

❖ **General Protocol**

Ensure that RNase A solution has been added to Resuspension Buffer. It is essential to completely resuspend the cell pellet. It may affect the lysis efficiency. Long exposure to alkaline condition may cause the plasmid to become irreversibly denatured. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps. Do not vortex, it may cause shearing of genomic DNA. If the Lysis Buffer is stored under the cold condition SDS precipitation may occur. It may cause the poor cell lysis. Therefore, before using the Lysis Buffer, warm it in 37℃ water bath to dissolve the SDS.

❖ **Growth of Bacterial Cultures**

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotics. The yield and quality of plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, antibiotics, and type of culture medium. High-copy number plasmids and large quantities of recombinant proteins can severely hamper the growth, and even the survival, of transformed cells. To prevent the emergence of bacteria from which the plasmid has been eliminated, it is important to sustain selective pressure by including the appropriate antibiotic in the culture medium at all times.

❖ **Plasmid Copy Numbers**

Plasmids vary widely in their number per cell, depending on their origin of replication (e.g., pMB1, ColE1, or pSC101) which determines whether they are under relaxed or stringent control; and depending on the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations which allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 and cosmids are generally present in lower copy numbers. Very large plasmids and cosmids are often maintained at very low copy numbers per cell.

DNA construct	Origin replication	Copy number	Classification
Plasmids			
pUC vectors	pMB1	500~700	High copy
pBluescript vectors	ColE1	300~500	High copy
pGEM® vectors	pMB1	300~400	High copy
pTZ vectors	pMB1	> 1000	High copy
pBR322 and derivatives	pMB1	15~20	Low copy
pACYC and derivatives	p15A	10~12	Low copy
pSC101 and derivatives	pSC10	1~5	Very low copy
Cosmids			
SuperCos	ColE1	10~20	Low copy
pWE15	ColE1	10~20	Low copy

TROUBLE SHOOTING GUIDE

This troubleshooting guide may be helpful in solving problems that may frequently arise. The scientists at iNtRON are always happy to answer any questions you may have about the information or protocol in this manual or other molecular biology applications.

Problem / Possible cause	Recommendation
Low yield	
1) Insufficient lysis of *E. coli* cells	• Insufficient lysis of *E. coli* cells decreases plasmid yield.
2) Poor quality of starting material or incomplete lysis	• Ensure media is completely removed after cell harvest. Or decrease the amount of starting material used.
3) Elution conditions require optimization	• If you are using a different buffer for elution, ensure that the pH of the buffer is 8.5 - 9.0.
4) Column was overloaded with DNA	• Check the culture volume and yield for use, and reduce the culture volume accordingly.
Low DNA quality	
1) Incomplete neutralization	• Incomplete neutralization generates poor quality supernatant and results in loading too much cell debris into the tube. Ensure that neutralization is complete by inverting the sample an additional 2-3 times after the addition of Lysis Buffer.
2) Overgrown culture	• Older cultures may contain more genomic DNA contamination than fresh cultures.
3) Genomic DNA in eluate	• Vortexing or overmixing after addition of the Lysis Buffer, Neutralization Buffer. Do not vortex samples after addition of the Lysis Buffer and Neutralization Buffer to prevent shearing of genomic DNA.
Degradation of purified plasmid	
1) Residual DNase	• Use DNase-gene deficient E. coli strains (e.g., DH5α). Plasmids from E. coli strain carrying DNase-gene (e.g., HB101) might be degraded during incubation.
Genomic DNA contamination	
1) Genomic DNA sheared during handling	• Gently invert the tubes to mix after adding buffers. **Do not vortex** as it can shear the genomic DNA. To efficiently precipitate the genomic DNA away from the plasmid DNA, the genomic DNA must be intact.
2) Degraded DNA	• Make sure that your entire equipment (pipettes, centrifuge tube, etc.) is clean and nuclease-free.
RNA contamination	
1) RNase A digestion omitted	• Ensure that RNase A is added to Resuspension Buffer before use.
2) RNase A digestion insufficient	• Check the Kit contests and storage; Resuspension Buffer must be stored at 4℃ after adding RNase A solution.
Plasmid DNA degradation	
1) Incorrect lysis procedure	• Incubate the lysate at room temperature for no longer than 5 minutes, because it might begin to denature the DNA

ORDERING INFORMATION

Product Name	Amount	Cat. No.
MEGAquick-spin™ Total Fragment DNA Purification Kit	50/200 col.	17286/17287/17288
DNA-spin™ Plasmid DNA Purification Kit	50/200 col.	17096/17097/17098
DNA-midi™ GT Plasmid DNA Purification Kit	25 col.	17254
MacCell™ DH5α 10^7 / 10^8 / 10^9	1 ml	15052/15053/15054
MacCell™ TOP10 10^7 / 10^8 / 10^9	1 ml	15055/15056/15057
Rapid MacCell™ DH5α Competent Cell	1 ml	15062
MacCell Express™ DH5α Competent Cell	1ml	15064
Maxime™ PCR PreMix (i-Taq)	96 tubes	25025
Maxime™ PCR PreMix (i-StarTaq)	96/480 tubes	25165/25167
Maxime™ PCR PreMix (i-MAX Ⅱ)	96 tubes	25265
Maxime™ PCR PreMix (i-StarTaq™ GH)	96 tubes	26050/26051
Maxime™ PCR PreMix (i-StarMAX™ GH)	96 tubes	26060

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Review date : 2017. 4. 20