DNA-spin™ Plasmid DNA Purification Kit

RUO | REF 17096 | Σ 50 | REF 17098 | Σ 200 | RT

The Instruction Manual for Plasmid DNA Extraction from E. coli using alkali lysis method and silica membrane.

DESCRIPTION

DNA-spin™ Plasmid DNA Purification Kit provide a fast, efficient means of preparing high purity plasmid DNA without specialized devices or equipment.

This kit contains a spin-type column filled with silica bead membrane and reagents optimizing alkali lysis for easy purification of plasmid DNA from bacteria. DNA-spin™ columns have a specialized silica gel membrane that binds up to 35 μg (maximum) DNA in the presence of a high concentration of chaotropic salt, and allows elution in a small volume of low-salt buffer. The membrane technology eliminates time consuming phenol-chloroform extraction and alcohol precipitation, as well as the problems and inconvenience associated with loose resins and slurries.

High-purity plasmid DNA eluted from DNA-spin™ columns is immediately ready to use - there is no need to precipitate, concentrate, or desalt.

CHARACTERISTICS

- High Quality : Highly purified plasmid DNA available to our specially treated plasmid DNA-specific silica bead membrane. Minimal nicks of plasmid DNA guarantees good results in plasmid DNA sequencing.
- Improved Recovery : Improved the DNA extraction yields - from short length (3 Kb) to long length plasmid (34 Kb)
- Prevention of error : Using a simple visual identification system, LysisViewer prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, cell debris, and genomic DNA.
- Fast : Takes only 30 minutes to extract plasmid DNA.

KIT CONTENTS

Label	17096 (50 Col)	17098 (200 Col)
Resuspension Buffer [1]	15 ml	55 ml
Lysis Buffer [2]	15 ml	55 ml
Neutralization Buffer [3]	20 ml	80 ml
Washing Buffer A [4]	30 ml	140 ml
Washing Buffer B [5]	10 ml	40 ml
Elution Buffer [6]	20 ml	20 ml
DNA-spin™ column [7]	50 columns	200 columns
Collection tube [8]	50 tubes	200 tubes
RNase A (Lyophilized powder) [9]	9 mg	33 mg
LysisViewer [10]	60 μl	220 μl

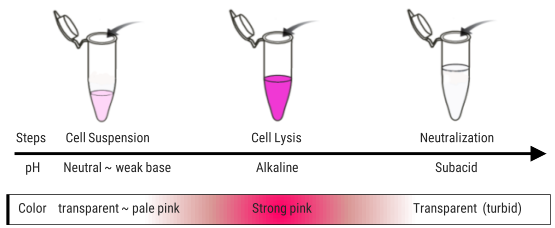
1) Before use, add reconstituted RNase A solution to Resuspension Buffer. Then, store at 4℃.
2) ⚠ Check Lysis Buffer for SDS precipitation due to low storage temperature, in which case it is necessary to dissolve the SDS by warming to 37℃.
3) This buffer contains acetic acid and chaotropic salt.
4) ⚠ endA+ strains such as HB101, the JM series strains, PR series strains and some other wide-type strains have high endonucleases activity. Endonucleases that can degrade plasmid DNA are essentially removed by Washing Buffer A of DNA-spin™ Kit.
5) ⚠ Before use, add 40ml (160ml) of absolute EtOH to the washing buffer before use.
6) DNase / RNase free Ultra-Pure solution.
7) The Columns containing silica membrane
8) Polypropylene tube for 2ml volume
9) The amount of lyophilized RNaseA provided is sufficient for the volume of Resuspension Buffer supplied with the kit. After receiving the kit, dissolve the lyophilized enzyme with Pure DW, then mix with Resuspension Buffer.
10)LysisViewer can be added to the Resuspension buffer bottle before use. Alternatively, smaller amounts of LysViewer can be added to aliquots of Resuspension buffer, enabling single plasmid preparations incorporating visual lysis control to be performed. LysisViewer should be added to Resuspension buffer at a ratio of 1:250 to achieve the required working concentration

STORAGE

DNA-spin™ Plasmid DNA Purification Kit should be stored at room temperature (15–25 °C). Under these conditions, DNA-spin™ Plasmid DNA Purification Kit can be stored for up to 24 months without showing any reduction in performance and quality. The RNase A is shipped at room temperature and should be stored immediately upon receipt at 2–8 °C.

LYSISVIEWER

◈ Color Change of Alkaline Lysis Step with LysisViewer



- LysisViewer can be added to the Resuspension Buffer bottle before use. Alternatively, smaller amounts of LysisViewer can be added to aliquots of Resuspension Buffer, enabling single plasmid preparations incorporating visual lysis control to be performed.
- The plasmid preparation procedure is performed as usual. After addition of Lysis Buffer to Resuspension Buffer, the color of the suspension changes to pink. Mixing should result in a homogeneously colored suspension. If the suspension contains localized regions of colorless solution or if cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.
- Upon addition of Neutralization Buffer, LysisViewer turns colorless. The presence of a homogeneous solution with no traces of pink indicates that SDS from the lysis buffer has been effectively precipitated.

ADDITIONAL REQUIRED EQUIPMENT

- Absolute ethanol
- Microcentrifuge tubes, sterile (1.5 ml)
- Standard tabletop microcentrifuge

APPLICATIONS

- Ligation and transformation
- In vitro translation
- Transfection of robust cells
- Restriction enzyme digestion
- Sequencing
- Library screening

TECHNICAL ASSISTANCE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are 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 DNA-spin™ Plasmid DNA Purification Kit or iNtRON products in general, please do not hesitate to contact us.

CONSIDERATION BEFORE USE

- ⚠ Lyophilized RNase A : Dissolve the RNase A in 0.9 ml (3.3 ml for 200 Tests) of pure D.W. For long-term storage of reconstituted RNase A, remove the stock solution from the vial, divide it into single-use aliquots, and store at −20 °C for up to 2 months. Thawed aliquots can be stored at 2–8 °C for up to 12 weeks. Do not refreeze the aliquots after thawing
- ⚠ If water is used for elution, make sure that its pH is between 7.0 and 8.5. Elution efficiency is dependent on pH and the maximum elution efficiency is achieved within this range. A pH <7.0 can decrease yield.
  Note: Store DNA at −20 °C when eluted with water, as DNA may degrade in the absence of a buffering agent.
- Lysis, Neutralization and Washing A Buffers contain irritants. Wear gloves when handling these buffers.

QUALITY CONTROL

- In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of DNA-spin™ Plasmid DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.
- The quality of the isolated plasmid DNA was checked by restriction analysis, agarose gel electrophoresis, and spectrophotometric determination.
- DNA-spin™ column control
  The DNA binding capacity was tested by determining the recovery obtained with 20 μg of input high-copy−plasmid DNA. More than 70% recovery was obtained.
- RNase A
  Tested in plasmid purification. At concentrations up to 10 μg per mL, nicking or degradation of plasmid is not detectable. One unit catalyzes the hydrolysis of RNA to yield an initial velocity constant of 1.0 at 25 °C, pH 5.0
- Buffer control
  Conductivity and pH of buffers were tested and found to be within the range below.

Buffer	Conductivity	pH
Resuspension	4.3 ~ 4.8 mS/cm	7.6 ~ 8.2
Lysis	39 ~ 45 mS/cm	10.9 ~ 11.5
Neutralization	154 ~ 170 mS/cm	2.8 ~ 3.1
Washing A	54 ~ 60 mS/cm	3.6 ~ 4.0
Washing B	11 ~ 13 mS/cm	7.4 ~ 7.8
Elution	550 ~ 620 μS/cm	8.0 ~ 8.5

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iNtRON Biotechnology
(# 701~704, Jung-Ang Induspia V, Sangdaewon-dong)
137, Sagimakgol-ro, Jungwon-gu, Sungnam-si, Gyeonggi-do, Korea

(주)인트론바이오테크놀로지
경기도 성남시 중원구 사기막골로 137
(상대원동, 중앙인더스피아 5차 701~704 호)

Customer & Technical Service
Do not hesitate to ask us any question
shop.intronbio.com
Tel : +82-505-550-5600
Fax : +82-505-550-5660
Mail : intronbio@intronbio.com

Instruction For Use
English (영문, 英語)
Belief & Relief, LiliF Diagnostics MDx Kit

PROTOCOL

- Add the dissolved RNase A solution to Resuspension Buffer, mix, and store at 2~8 ºC.
- Add ethanol (96–100%) to Washing Buffer B before use (see bottle label for volume).
- Check Lysis Buffer and Neutralization Buffer before use for salt precipitation. Redissolve any precipitate by warming to 37 ºC. Do not shake Lysis Buffer vigorously.
- Optional: Add the provided LysisViewer to Resuspension Buffer and mix before use. Use one vial of LysisViewer (spin down briefly before use) per bottle of Resuspension Buffer to achieve a 1:250 dilution. LysisViewer provides visual identification of optimum buffer mixing thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris.

1. Pick a single colony from a freshly streaked bacterial plate and use it to inoculate LB (+antibiotics). And then grow at 37 ℃ for 12 ~ 16 hrs with vigorous shaking (OD600 = 1.0 ~ 1.5).
2. Harvest 3 – 5 ml of bacteria culture by centrifugation at 13,000 rpm for 30 sec at RT and discard supernatant.
   Note : Drain tubes on a paper towel to remove excess media.
3. Resuspend pelleted bacterial cell thoroughly in 250 µl of Resuspension Buffer by vortexing until no clumps remain.
   Note : 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
4. Add 250 µl of Lysis Buffer to resuspended cells and mix by inverting the tube 10 times. DO NOT VORTEX and incubate for 3 min at RT.
   Note : The optimal lysis time allows maximum release of plasmid DNA without release of chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. 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.
   Note : If the Lysis buffer becomes too cold, SDS precipitation may occur, leading to poor cell lysis. If a precipitate has formed, warm the Lysis buffer to 37 ºC with gentle shaking.
5. Add 350 µl of Neutralization Buffer and gently mix by inverting the tube 10 times then incubate the tube in ice for 5 min.
   Note : After addition of Neutralization Buffer, the solution should become cloudy and a fluffy white form. Incubation on ice may help precipitating the denatured cell components more efficiently. The precipitated material contains genomic DNA, protein, cell debris, and SDS.
   Note : If LysisViewer reagent has been used, the suspension should be mixed until all trace of pink has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.
6. Centrifuge at 13,000 rpm for 10 min at 4℃. While waiting for the centrifugation, insert a column into collection tube.
7. After centrifugation, transfer supernatant promptly into the column.
   Note : Cell debris, protein and genomic DNA will form a compact white pellet in the tube. Do not transfer with white pellet.
8. Centrifuge at 13,000 rpm for 1 min. Remove the column from collection tube, discard filtrate in collection tube. And then place the spin column back in the same collection tube.
9. (Optional) Add 500 µl of Washing Buffer A and centrifuge at 13,000rpm for 1 min. Remove the column from collection tube, discard filtrate in collection tube. And then place the spin column back in the same collection tube.
   Note : This step is necessary to remove trace nuclease activity. endA+ strains, such as BL21, HB101, JM series, or any wild-type strains, have high level of nuclease activity that can degrade plasmids. But endA-strains,such as DH5α, XL1-blue and etc, do not require this additional washing step.
10. Add 700 µl of Washing Buffer B, centrifuge at 13,000 rpm for 1 min. Discard filtrate in the collection tube and place the spin column back in the same collection tube.
    Note : If the DNA will be used for salt sensitive applications, such as blunt-end ligation and direct sequencing, repeat the step10 using 500 µl of Washing Buffer B.
11. Centrifuge at 13,000 rpm for 1 min to dry the filter membrane.
    Note : Completely remove ethanol. Residual ethanol from Washing Buffer B may inhibit subsequent enzymatic reaction.
12. Put the column into a clean and sterile centrifuge tube. Add 50 µl of Elution Buffer or distilled water to the upper reservoir of the column, and let it stand for 1min. Then, centrifuge the tube assembly at 13,000 rpm for 1 min.
    Note : It is suggested to use at least 30 µl of the Elution buffer to obtain best result. If the plasmid is low-copy number or larger than 10 Kb, the yield of plasmid may not be sufficient. In this case, pre-warmed (about 50℃) elution buffer will improve efficiency of elution.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low or no yield	Plasmid did not propagate	• Check that the conditions for optimum growth were met
	Lysis buffer is precipitated	• Check the Lysis buffer for SDS precipitation due to low storage temperature and dissolve the SDS by warming to 37oC.
	Lysis buffer incompletely mixed	• Ensure complete mixing all buffers, when put and mix Lysis buffer and Neutralization buffer, do not mix strongly
	Cell resuspension Incomplete	• Pelleted cells should be completely resuspended in Resuspension buffer. Do not add Lysis buffer until an even suspension is obtained.
	Step were not followed correctly or wrong reagent used	• Check the protocol; Washing buffer did not contain 100% EtOH, so, 100% EtOH must be added to the Washing buffer before use.
	Plasmid did not propagate	• Check the bacterial culture conditions (age of culture, antibiotics, culture volume and bioreactor)
RNA in the eluate	RNase A digestion omitted	• Ensure that RNase A is added to Resuspension Buffer before use
	RNase A digestion insufficient	• Reduce culture volume if necessary. If Resuspension Buffer containing RNase A is more than 6 months old, add additional RNase A
	RNase A digestion insufficient	• Check the KIT CONTENT and STORAGE; Resuspension buffer shall be stored at 4℃ after adding RNase A solution. • Increase the incubation time after mixing with Neutralization Buffer for 3~5min
DNA is nicked /sheared/degraded	Endonuclease-containing host	• Because it is different that have endonuclease-containing in host strain, consider changing E.coli host strain. • When put and mix Lysis buffer and Neutralization buffer, do not shake strongly
Genomic DNA In the eluate	Lysis time was too long	• Ensure that the lysis step does not exceed 5min.
	Lysis Buffer added incorrectly	• The Lysate must be handled gently after addition of Lysis Buffer to prevent shearing. Reduce culture volume if lysate is too viscous for gentle mixing
	Neutralization Buffer added incorrectly	• Upon addition of Neutralization Buffer, mix immediately but gently
	Culture overgrown	• Overgrown cultures contain lysed cells and degraded DNA. Do not grow cultures for longer than 12~16 hours

EXPERIMENTAL INFORMATION

◈ Yields of various sizes of plasmid DNA

DNA-spin™ Kit is shown highly improved efficiency of plasmid DNA Recovery from high copy plasmid DNA and low copy plasmid DNA.

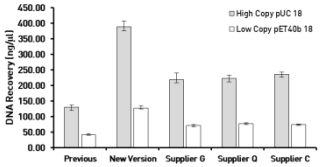

Fig. 1. Yield of plasmid DNA
Plasmid DNAs were extracted from 5 ml (OD600 of 1.0) of E. coli cultures containing pUC 18 (app. 2.7kb) and pET40b (app. 6.2 kb). Newly developed DNA-spin Plasmid DNA Purification Kit is shown improved DNA extraction recovery as 1.5 ~ 3 folds compared with previous version kit and other suppliers', spectrophotometically.

◈ Improved column binding capacity

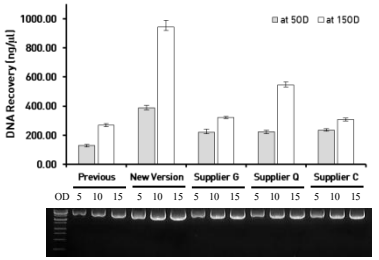

Fig. 2. Comparative test of the DNA recovery rate according to the sample start amounts. pUC18 plasmid DNA were isolated from 5~15 OD_{600} as sample start amount. As the results, the newly deveoped DNA-spin kit was shown 1.5~2.5 fold improved recovery compared with previous kit and other supplies. In case of newly developed DNA-spin kit was observed up to 45 µg of maximal recovery.

◈ Suitable for Down-stream Operations

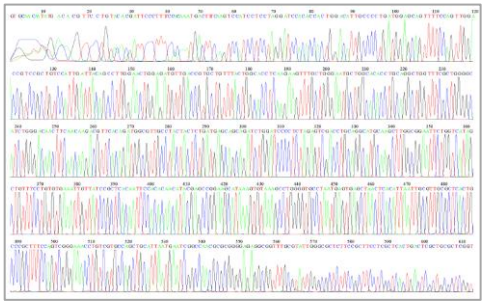

Fig. 3. Reliable Long Read Lengths in Sequencing
High quality sequencing data of pUC18 clones purified with iNtRON's DNA-spin™ Kit.

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EXPLANATION OF SYMBOLS

Manufactured by
Attention
Consult Instructions For Use
Manufacturing date
Expire date
Keep away from sunlight
Sufficient for tests
Do not reuse
Storage temperature limitation
LOT Batch number
RUO Research Use only
REF Product number

문서번호 :
작성일자 :