For general laboratory use

Gene Transfer Pioneers
(GTP)

Plasmid DNA Isolation Kit

Kit for 100 purifications

Catalogue Number: DV01100

Store the kit at +15 to +25°C.

Introduction

The **Plasmid DNA Isolation Kit** was designed for rapid isolation of plasmid DNA from 1-7 ml of cultured bacterial cells. A modified alkaline lysis method and RNase treatment are used to obtain clear cell lysate with minimal genomic DNA and RNA contaminants. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 15 minutes. The purified plasmid DNA is ready for use in restriction enzyme digestion, ligation, PCR, and sequencing reactions.

Quality Control

The quality of the **Plasmid DNA Isolation Kit** is tested on a lot-to-lot basis by isolating plasmid DNA from a 4 ml overnight E. coli (DH5α) culture containing plasmid pBluescript (A600 > 2 U/ml). Following the purification process, a yield of more than 30 µg is obtained and the A260/A280 ratio is between 1.8-2.0. The purified plasmid DNA (1 µg) is used in EcoRI digestion, and analyzed by electrophoresis.

Kit Contents

All solutions are clear. If any solution contains a precipitate, do not use it. Instead, warm the solution at room temperature or in a 37°C water bath to dissolve the precipitate.

Vial	Label	Contents
1. White Cap	PL1 Buffer	40 ml
2. White microtube	RNase A	50 µl
3. Red Cap	PL2 Bufer	40 ml
4. Brown Cap	PL3 Buffer	40 ml
5. Blue Cap	Wash Buffer	12 ml; add 48 ml ethanol
6. Black Cap	Elution Buffer	20 ml
7. Colorless Bag	DNA Spin Column	100 Columns

Storage and Stability

Store the Plasmid DNA Extraction Kit components at +15 to +25°C. Kit components are guaranteed to be stable date printed on the label.

After adding RNase A, store the Suspension Buffer at +2 to +8°C, where it will be stable for 6 months.

Application

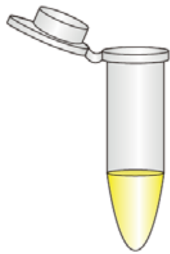
Isolation of up to 15 µg purified plasmid DNA from bacterial culture, which may be used directly in downstream applications such as restriction enzyme digestion, PCR, cloning, sequencing, *in vitro* transcription or labeling reactions.

Before You Begin: Preparation of Working Solutions

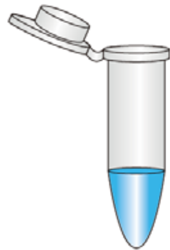
Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solution:

Content	Preparation	Storage/ Stability
RNase A / PL1 Buffer (White Cap)	Add all 50 µl of RNase A into the PL1 Buffer bottle. * Label bottle accordingly after adding RNase A.	Store the mixture at +2 to +8°C. Stable for 6 months.
Wash Buffer (Blue Cap)	Add 48 ml absolute ethanol to Wash Buffer. * Label bottle accordingly after adding ethanol.	Store at +15 to +25°C. Stable through expiration date printed on kit label.

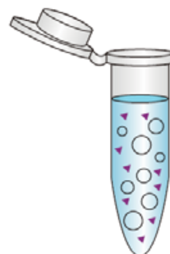
Quick Protocol Diagram



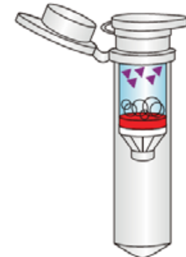
Harvest cultured bacterial cells by centrifuge to form a cell pellet, followed by resuspension



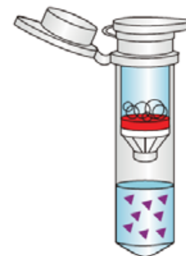
Lyse bacterial cells (Do not vortex)



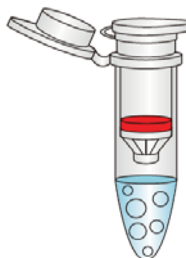
Neutralize suspension (Do not vortex)



DNA binding to membrane while contaminants remain suspended



Wash (removal of contaminants while DNA remains bound to membrane)



Elution of pure plasmid DNA which is ready for subsequent reactions

Purification of DNA Plasmid

1. Pellet the bacterial cells from 1-4 ml of E. coli culture by centrifuge in 8000-10000 g for 1 min. Discard the supernatant.

2. Add 250 µl **PL1 Baffer** (**White Cap**)(with RNase A) to the bacterial pellet. Resuspend the bacterial pellet by vortex for 30 s.

3. Add 250 µl **PL2 Baffer** (**Red Cap**) to the mixure. Mix gently by inverting the tube 5 to 10 times (**Do not vortex**). Incubated at room temperature for 4-5 min.

4. Add 350 µl **PL3 Baffer** (**Brown Cap**). Mix gently by inverting the tube 5 to 10 times (**Do not vortex**). Incubated on ice for 4-5 min (The solution should become cloudy and a flocculent precipitate should form).

5. Centrifuge for 10 min at 8000-10000 g at room temperature.

6. After centrifugation, transfer intiresupernatant into DNA spin column.

7. Centrifuge for 1 min at 8000-10000 g at room temperature.

8. Disconnect the Filter Tube, and discard the flowthrough solution. Reconnect the Filter Tube to the same Collection Tube.

9. Add 500 µl **Wash Buffer** (**Blue Cap**) to DNA spin column.

10. Centrifuge 1 min at maximum speed (8000 – 10000 g). Discard the flowthrough solution.

11. Centrifuge the DNA spin column at 10.000 g for 1 min to remove residual ethanol.

12. Reconnect the Filter Tube to a clean 1.5 ml microcentrifuge tube.

13. Add 50 µl pre-warmed **Elution Buffer** (**Black Cap**) or sterile DDW directly onto column membrane and stand for 3 min.

14. Centrifuge at 10.000 g for 1 min.

15. Add another 50 µl pre-warmed **Elution Buffer** (**Black Cap**) or sterile DDW directly onto column membrane and stand for 3 min.

16. Centrifuge at 10.000 g for 1 min.

17. Store DNA at 4˚C or -20˚C.

Troubleshooting

Low Yield

Incomplete buffer preparation.

For **Plasmid DNA Isolation Kit** add provided RNase A to PL1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8ºC for up to 6 months. If precipitates have formed in PL2 Buffer, warm in a 37ºC water bath followed by gentle shaking to dissolve. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Incomplete cell culture preparation.

We recommend using a single freshly isolated *E. coli* colony to inoculate into 1-10 ml of LB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures (≤16 hours incubated in a culture tube at 37ºC with 150-180 rpm shaking).

Culture growth medium was not removed completely.

Following centrifugation in the harvesting step, use a narrow pipette tip to ensure the supernatant is completely removed.

Cell pellet was not resuspended completely.

Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

Bacterial cells were not lysed completely.

Using 2 - 6 OD600 units of bacterial culture is recommended. Do not vortex to avoid shearing the genomic DNA.

Incorrect DNA Elution step.

Ensure that Elution Buffer, TE or water is added into the center of the DNA Spin Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer, TE, or water (60~70ºC). If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

No yield of plasmid DNA.

Increase volume of low-copy number plasmid to 5-7 ml. We recommend using a single freshly isolated E. coli colony to inoculate into 1-10 ml of LB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures. Use fresh cultures only.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Following the Wash Step, dry the DNA Spin Column with additional centrifugation at 14000 x g for 5 minutes.

Residual salt contamination.

Perform the Wash Step twice for salt sensitive downstream applications.

RNA contamination.

Add provided RNase A to PL1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8ºC for up to 6 months. After adding PL2 Buffer to the sample mixture, mix gently by inverting the tube 10 times then let stand at room temperature for 2-5 minutes.

Genomic DNA contamination.

Do not use overgrown bacterial cultures. Use only fresh cultures as they will contain less genomic DNA than old cultures. During PL2 and PL3 Buffer addition, mix gently to prevent genomic DNA shearing.

Related DNA Extraction Products

Product	Package Size	Catalogue Number
Plasmid DNA Isolation Kit	25 preps	DV01025
Plasmid DNA Isolation Kit	50 preps	DV01050
Plasmid DNA Isolation Kit	100 preps	DV01100
DNA Gel Recovery Kit (PCR Purification Kit)	25 prep	DR02025
DNA Gel Recovery Kit (PCR Purification Kit)	50 preps	DR02050
Genomic DNA Extraction Kit from Gram Negative Bacteria	25 prep	DM04025
Genomic DNA Extraction Kit from Gram Negative Bacteria	50 prep	DM04050
Genomic DNA Extraction Kit from Gram Positive Bacteria	25 prep	DM05025
Genomic DNA Extraction Kit from Gram Positive Bacteria	50 prep	DM05050
Genomic Yeast DNA Extraction Kit	25 prep	DY06025
Genomic Yeast DNA Extraction Kit	50 prep	DY06050
Plant Total DNA Extraction Kit	50 prep	DP07050
Plant Total DNA Extraction Kit	100 prep	DP07100
Genomic DNA Extraction Kit from Seed	50 prep	DS08050
Genomic Fungi DNA Extraction Kit	25 prep	DF09025
Genomic Fungi DNA Extraction Kit	50 prep	DF09050
Blood DNA Extraction Kit	25 prep	DB10025
Blood DNA Extraction Kit	50 prep	DB10050
Blood DNA Extraction Kit	100 prep	DB10100
Tissue & Cells DNA Isolation Kit	50 prep	DT11050

www.irgtp.com
Tel: 021 – 88200114