# PLASMID DNA EXTRACTION MINI KIT

## Description

The DNA Plasmid Extraction Kit provides a rapid, phenol-free method for the extraction of high-purity plasmid DNA from bacterial cultures such as E. coli , which bacteria is pellet, lysed, and then neutralized. The extracted DNA can be used in a variety of applications such as PCR, cloning, sequencing, in vitro transcription, and labeling. Also, as a column-type tube is utilized in the purification process, extraction is carried out in three simple steps of binding / washing / elution. Once bound, the DNA is washed and then eluted from the column, ready for use.

## Features

- For high yields of plasmid DNA-up to 30µg from 1~5ml overnight cultures.
- Effective purification of DNA fragments ranging from 100bp to 12+kb.
- No need for messy resin slurries, extracting with phenol, or concentrating via alcohol precipitation.
- Superior purity-DNA yields quality sequence data using automated or manual methods.
- Optimized buffers are included for maximum DNA purity and yield.
- Versatile protocol-works with all neutral gel buffers and both conventional and low-melting agarose gel.

## Format

Spin Columns

## Operation

Centrifuge/vacuum

## Binding Capacity

Up to 30 µg

## Expectant Yield

20-30 µg for high-copy plasmid
3-10 µg for low-copy plasmid

## Time Required

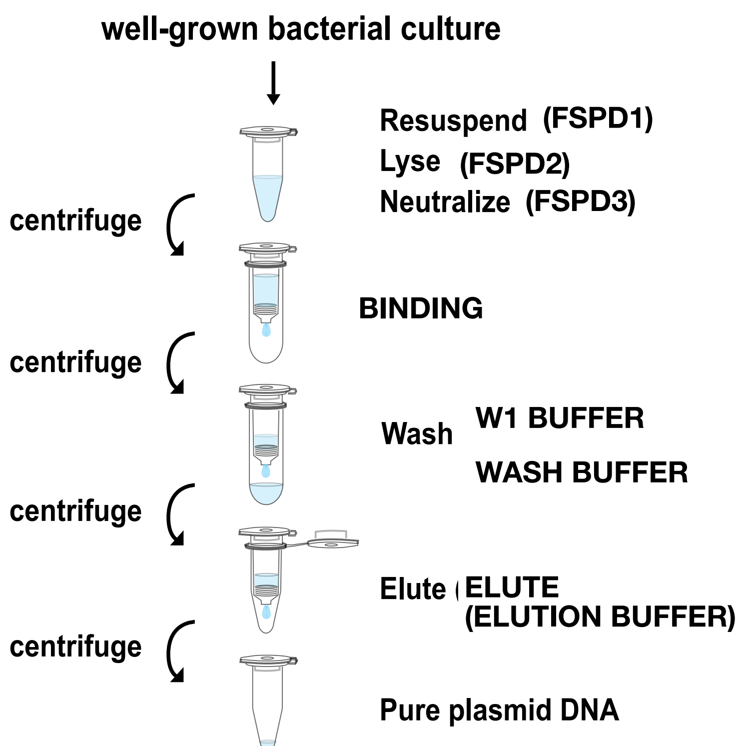
Under 30 minutes

## Applications

- Fluorescent or radioactive Sequencing
- Ligation
- Restriction enzyme digestion
- Ligation and Transformation
- Library screening

## Time Required

Under 30 minutes

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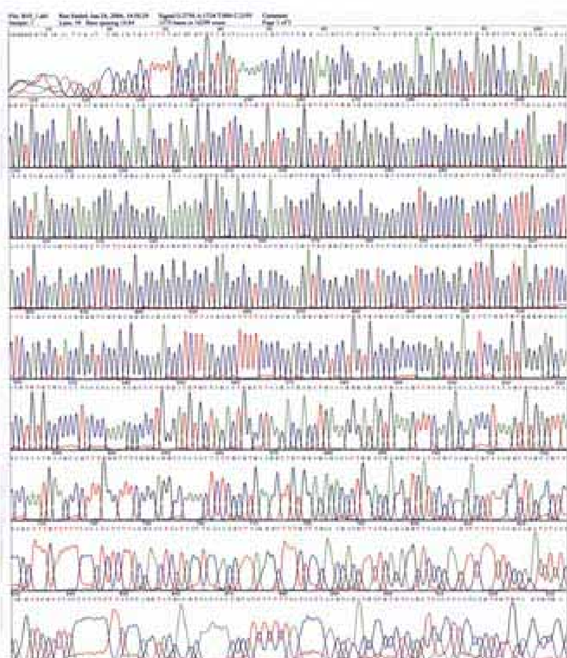
## DNA Sequencing Analysis

Sequencing of plasmid purified with Plasmid DNA Extraction Mini Kit. The sequence was analyzed with BigDye Terminator chemistry on ABI 3700



## Ordering Information

| Cat. No. | Product Name | Size | Kit Components | Store at |
|---|---|---|---|---|
| DE-005 | Plasmid DNA Extraction Kit | 100 preps. | 1. FSPD 1 Buffer<br>2. FSPD 2 Buffer<br>3. FSPD3 Buffer<br>4. W1 Buffer<br>5. Wash Buffer (conc.)<br>6. Elution Buffer<br>7. RNase A (50 mg/ml)<br>8. FSPD Columns<br>9. 2 ml Collection Tube | FSPD 1 Buffer with RNaseA at 4 °C, other components should at room temperature (15 -25 °C ). |
| DE-006 | Plasmid DNA Extraction Kit | 300 preps. | 1. FSPD 1 Buffer<br>2. FSPD 2 Buffer<br>3. FSPD3 Buffer<br>4. W1 Buffer<br>5. Wash Buffer (conc.)<br>6. Elution Buffer<br>7. RNase A (50 mg/ml)<br>8. FSPD Columns<br>9. 2 ml Collection Tube | FSPD 1 Buffer with RNaseA at 4 °C, other components should at room temperature (15 -25 °C ). |

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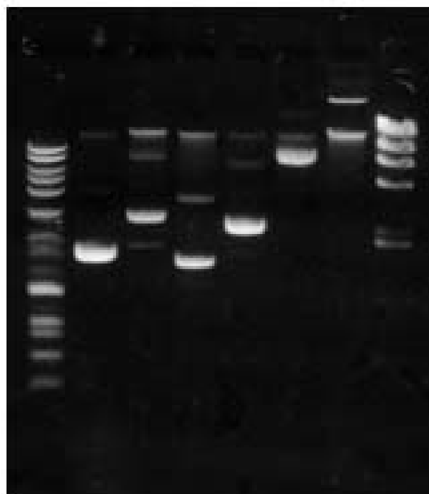


**Agarose Gel Analysis Of Plasmids Purified**

Agarose gel analysis of 5 plasmid and cosmid extracted by the Plasmid DNA Extraction Mini Kit.

M: 1kb DNA ladder (100 bp-12,000 bp)
1: pBluescript II
2. pUC 18
3: pBR 322
4: pGem with 1 Kb insert
5: pET 43.1 with 2.7 Kb insert
6: cosmid (50 Kb)
M1: 1 Kb DNA Ladder
M2: Lambda-HindIII

## Procedure

The modified alkaline lysis method and RNase treatment are used for cteating cleared cell lysate with minimal genomic DNA and RNA contaminants. In the presence of a chaotropic salt, the plasmid DNA in the lysate binds to the glass fiber matrix in the spin column. The purified plasmid DNA is eluted by a low salt elution buffer or water. The procedure doesn't require DNA phenol extraction or alcohol precipitation.

## Storage Conditions

Plasmid DNA Extraction Kit can be stored at room temperature (15-25°C). After adding RNase A, FSPD Buffer1, should be stored at 4°Cand is stable for six months. Other buffers and columns can be stored dry for up to 1 year at room temperature (15-25°C).

Distributed by:

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*Protocol*

## Introduction

Plasmid Extraction Mini Kit is an excellent tool offering a speed and economic method to purify plasmid DNA from bacteria cultures. This technology is based on binding DNA to silica-based membranes in chaotropic salts, washing DNA with ethanol-contained Wash Buffer. Compare with other harmful and time-consuming procodure, such as phenol/ chloroform extraction and ethanol precipitation, Plasmid extraction kit shortens the handling time to about 25 minutes. The high quality plasmid DNA can be used directly for the downstream application.

## Specification

Sampling: 1~5 ml overnight culture
Plasmid Size: < 12Kb
Yield: 20~30 µg of high-copy plasmid
Handing time: about 25 min

| Kit Contents: | DE-005 | DE-006 |
|---|---|---|
| FSPD1 Buffer | 30 ml | 90 ml |
| FSPD2 Buffer | 30 ml | 90 ml |
| FSPD3 Buffer | 40 ml | 120 ml |
| Wash Buffer (concentration)** | 20 ml | 50 ml |
| W1 Buffer (concentration)* | 35 ml | 98 ml |
| Elution Buffer | 15 ml | 35 ml |
| RNase A (50mg/ml) | 60 µl | 180 µl |
| FSPD Column | 100 pcs | 300 pcs |
| Collection Tube | 100 pcs | 300 pcs |
| User Manual | 1 | 1 |

* Add 13 ml/ 36 ml ethanol (96 ~ 100%) to W1 Buffer when first open.
** Add 80 ml/ 200 ml ethanol (96 ~ 100%) to Wash Buffer when first open.

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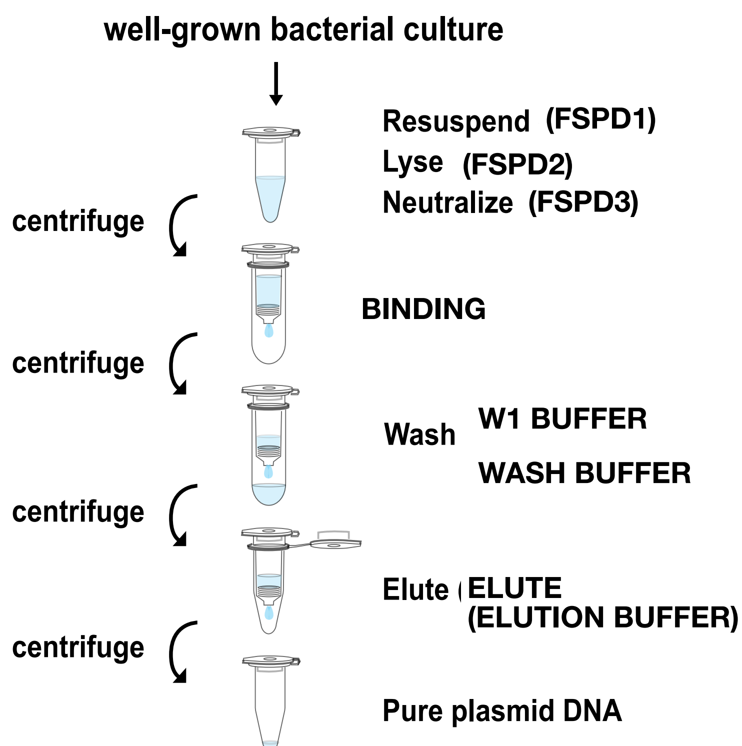
## Important Notes:

1. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffer.
2. Brief spin RNase A tube to remove drops from the inside of the lid. Add 1 ml of FSPD1 Buffer into RNase A tube and mix well. Transfer the mixture into FSPD1 Buffer bottle and store at 4 ° C.
3. Check FSPD2 Buffer before use. Warm FSPD2 Buffer at 55 ° C for 10 minutes if any precipitation formed. Don't shake FSPD2 Buffer vigorously.
4. To avoid acidification of FSPD2 Buffer from $CO_2$ in the air, close the bottle immediately after use.
5. For DE-005, add 13 ml ethanol (96~100%) to W1 Buffer when first open. For DE-006, add 36 ml ethanol (96~100%) to W1 Buffer when first open.
6. For DE-005, add 80 ml ethanol (96~100%) to Wash Buffer when first open. For DE-006, add 200 ml ethanol (96~100%) to Wash Buffer when first open.
7. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

## Brief Procedure



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## • General Protocol:

1. Transfer 1-5 ml of well-grown bacteria culture to a microcentrifuge tube (not provided).

2. Descend the bacteria by centrifuging for 1-2 min and discard the supernatant completely.

3. Add 250 µl of FSPD1 Buffer to the pellet and resuspend the cells completely by pipetting.
   - **Make sure that RNase A has been added into FSPD1 Buffer when first open.**
   - **No cell pellet should be visible after resuspension of the cells.**

4. Add 250 µl of FSPD2 Buffer and gently invert the tube 5 times to lyse the cells and incubate at room temperature for 2 min.
   - **Do not vortex, vortex may shear genomic DNA. If necessary, continue inverting the tube until the lysate beccome clear.**
   - **Do not proceed this step over 5 min.**

5. Add 350 µl of FSPD3 Buffer and invert the tube 5 times immediately but gently.
   - **Invert immediately after addind FSPD3 Buffer will avoid asymmetric precipitation.**

6. Centrifuge for 10 min. During centrifuging, place a FSPD Column in a Collection Tube.

7. Transfer the suspernatant carefully to FSPD Column. Centrifuge for 1 min then discard the flow-through.
   - **Do not transfer any white pellet into the column.**

8. Add 400 µl of W1 Buffer to FSPD Column. Centrifuge for 1 min then discard the flow-through.
   - **Make sure that ethanol (96-100 %) has been added into W1 Buffer when first open.**

9. Add 750 µl of Wash Buffer to FSPD Column. Centrifuge for 1 min then discard the flow-through.
   - **Make sure that ethanol (96-100 %) has been added into Wash Buffer when first open.**

10. Centrifuge for an additional 5 min to dry the column.
    - **Important step ! This step will remove the residual liquid completely that will inhibit subsequent enzymatic reaction.**

11. Place FSPD Column to a new 1.5 ml microcentrifuge tube (not provided).

12. Add 50 µl ~ 100 µl of Elution Buffer or $ddH_2O$ to the membrane center of FSPD Column. Stand the column for 1 min.
    - **Important step ! For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely.**

> • **Important : Do not elute the DNA using less than suggested volume (50µl). It will lower the final yield.**

13. Centrifuge for 1 min to elute plasmid DNA.

14. Store plasmid DNA at 4 °C or -20 °C.

## • Troubleshooting

***Low yield***

Bacterial cells were not lysed completely
- **Too many bacterial cells were used (OD600 > 10). Separate the bacterial culture into multiple tubes.**
- **After FSPD3 Buffer addition, break up the precipitate by inverting to ensure higher yield.**

Overgrown of bacterial cells
- **Incubation time should not longer than 16 hours.**

Bacterial cells were insufficient
- **Ensure that bacterial cells have grown to an expected amount (OD600 > 1) after incubation under suitable shaking modes.**

Incorrect DNA Elution Step
- **Ensure that Elution Buffer was added and absorbed to the center of FSPD Column Martix.**

Incomplete DNA Elution
- **If size of DNA fragments is larger than 10 kb, use preheated Elution Buffer (60~70°C) on Elution Step to improve the elution efficiency.**

Incorrect Wash Buffer
- **Ensure that Ethanol was added to Wash Buffer pior to use.**

***Eluted DNA does not perform well***

Residual ethanol contamination
- **After Wash Step, dry FSPD Column with additional centrifugation at top speed for 5 minutes or incubation at 60°C for 5 minutes.**

***Genomic DNA Contaminates***

Lysate prepared improperly.
- **Gently invert the tube after adding FSPD2 Buffer. And the incubation time should not longer than 5 minutes.**
- **Do Not use overgrown bacterial culture.**

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## • Troubleshooting

*RNA Contaminates Plasmid DNA*

Insufficiency of RNase A activity in FSPD1 Buffer because of long-term storage

- **Prior to using FSPD1 Buffer, ensure that RNase A was added. If RNase A added FSPD1 Buffer is out of date, add additional RNase A into FSPD1 Buffer to a concentration of 50µg/ml then store 4°C.**
- **Too many bacterial cells were used, reduce sample volume.**

*Smearing or degrading of Plasmid DNA*

Nuclease contamination

- **If used host cells have high nuclease activity (e.g., enA+ strains), perform this Optional Wash Step to remove residuary nuclease.**
- **After DNA Binding Step, add 400µl of W1 Buffer into FSPD column and column and incubate for 2 minutes at room temperature.**
- **Centrifuge at full speed (14,000 rpm or 10,000 xg) for 30 seconds.**
- **Followed using standard Wash Step.**

*Plasmid DNA is not adequate for enzymatic digestions*

Eluted plasmid DNA contains residual ethanol

- **Make sure you have discarded the flow-through after washing with Wash Buffer (Step 9) and centrifuged for an additional 3 minutes (Step 10).**

*Denatured Plasmid DNA migrate faster than supercoilded form during electrophoresis*

Incubation in FSPD2 Buffer is too long

- **Do not incubate longer than 5 minute in FSPD2 Buffer**



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