Yeastern Biotech Co., Ltd

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HiYield Plasmid Mini Kit 2.0

Cat. No.
FYG007-100P
FYG007-300P
SYG007-001

HiYield Plasmid Mini Kit 2.0

FYG007-100P (100 preps)

FYG007-300P (300 preps)

SYG007-001 (Sample)

Store at room temperature (15°C-25°C)

Sample	1-5 ml bacterial culture
Yield	up to 40 μg plasmid/cosmid DNA
Format	spin column
Operation time	up to 30 minutes
Elution volume	30-50 μl

Description

HiYield Plasmid Mini Kit 2.0 is specially designed for rapid isolation of plasmid or cosmid DNA from 1-5 ml of bacterial cultured cells. As high as 40 μg of high quality plasmid DNA can be purified in less than 30 minutes and is ready for restriction digestion, ligation, PCR, and sequencing reaction.

No phenol extraction or alcohol precipitation is required in this protocol. In the process, clear and extra pure cell lysate with minimal genomic DNA and RNA contaminants can be obtained through the modified alkaline lysis method and RNase treatment. In the presence of a chaotropic salt, the plasmid DNA within the lysate will then bind to the glass fiber matrix equipped in the spin column. The contaminants are washed away with an ethanol-containing wash buffer. Finally, the purified plasmid DNA is eluted by a low salt elution buffer or distilled water. Typical yields of high-purity are 20~40 μg for high-copy number plasmids or 3~10 μg for low-copy number plasmids.

Quality Control

The quality of **HiYield Plasmid Mini Kit 2.0** is tested on a lot-to-lot basis. The kit is tested by isolation of plasmid DNA from 5 ml culture of *E.coli* DH5α transformed with the plasmid pGAD424 (A_{600}>2 units/ml). More than 40 μg of plasmid DNA should be obtained. One μg of the purified product is also tested for restriction enzyme digested with *Eco*R I followed by agarose gel analysis.

Components

Component	SYG007-001 (Sample)	FYG007-100P (100 preps)	FYG007-300P (300 preps)
PDX1 Buffer[1]	1.5 ml (RNase A added)	24 ml	72 ml
PD2 Buffer[2]	2 ml	30 ml	90 ml
PDX3 Buffer	3 ml	40 ml	120 ml
WX1 Buffer	2.4 ml	60 ml	180 ml
Wash Buffer (Add EtOH)[3]	1 ml (4 ml)	20 ml (80 ml)	30+30 ml (120+120 ml)
Elution buffer	0.4 ml	10 ml	30 ml
RNase A (20 mg/ml)	---	84 μl	252 μl
PDX Column	4 pcs	100 pcs	300 pcs
Collection Tubes	4 pcs	100 pcs	300 pcs

[1] Add the provided RNase A to **PDX1 Buffer** and store at 4°C.

[2] If precipitates have formed in **PD2 Buffer**, warm the buffer at 37°C to dissolve.

[3] Add 4 ml (SYG007-001) / 80 ml (FYG007-100P) / 120 ml (FYG007-300P) of ethanol (96 ~100%) to **Wash Buffer** before first use.

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Protocol

Step 1. Harvesting

a. Transfer 1.5 ml of bacterial culture to a microcentrifuge tube (not provided).
b. Centrifuge for 1 min at full speed (14-16,000 x g) in a microcentrifuge and discard the supernatant.

(Repeat the Harvesting step if >1.5 ml of bacterial culture is used.)

Step 2. Resuspension

a. Add 200 µl of **PDX1 Buffer** (RNase A added) to the micorcentrifuge tube and resuspend the cell pellet by vortexing or pipetting.

Step 3. Lysis

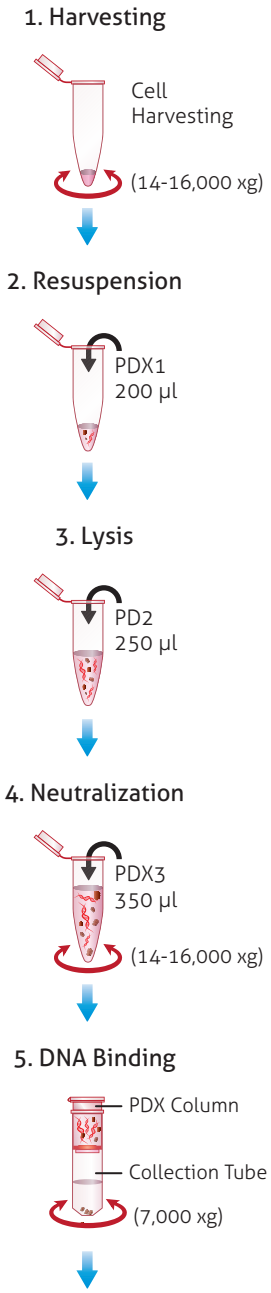
a. Add 250 µl of **PD2 Buffer** and mix gently by inverting the microcentrifuge tube for 10 times. *Do not vortex* to avoid shearing of genomic DNA.
b. Stand for 2~5 minutes at room temperature until lysate clears.

Step 4. Neutralization

a. Add 350 µl of **PDX3 Buffer** and mix immediately by inverting the tube 10 times. *Do not vortex.*
b. Centrifuge for 3 minutes at full speed (14-16,000 x g).

Step 5. DNA Binding

a. Place a **PDX Column** in a **2 ml Collection Tube**.
b. Apply the clear lysate (supernatant) from Step 4 to the **PDX Column**.
c. Centrifuge at 7,000 x g for 30 seconds.
d. Discard the flow-through and place the **PDX Column** back in the **Collection Tube**.



Step 6. Wash

a. Add 500 µl of **WX1 Buffer** in the **PDX Column**.
b. Centrifuge at 7,000 x g for 30 seconds.
c. Discard the flow-through and place the **PDX Column** back in the **Collection Tube**.
d. Add 700 µl of **Wash Buffer** (ethanol added) in the **PDX Column**.
e. Centrifuge at 7,000 x g for 30 seconds.
f. Discard the flow-through and place the **PDX Column** back in the **Collection Tube**.
g. Centrifuge again for 3 minutes at full speed (14-16,000 x g) to dry the column matrix.

Step 7. DNA Elution

a. Transfer dried **PDX Column** on a clean microcentrifuge tube (not provided).
b. Add 50 µl of **Elution Buffer** or water into the center of the column matrix.
c. Stand for 2 minutes until **Elution Buffer** or TE is absorbed by the matrix.
d. Centrifuge for 2 minutes at full speed (14-16,000 x g) to elute purified DNA.

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Troubleshooting

The yield is low

1. Bacterial Cells Were not Lysed Completely

» Too many Bacterial cells were used. If more than 10 OD $_{600}$ units of bacterial culture was used, separate it into multiple tubes.

» After the addition of **PDX3 Buffer**, break up the precipitate by inverting to ensure higher yield.

2. Incorrect DNA Elution Step

» Ensure that **Elution Buffer** was added and absorbed to the center of **PDX Column** matrix.

3. Incomplete DNA Elution

» If plasmid DNA are larger than 10 Kb, use preheated **Elution Buffer** (60~70°C) at Elution Step to improve the elution efficiency.

Eluted DNA does not perform well in downstream applications

1. Ethanol Residue contamination

» After Wash Step, dry **PDX Column** with additional centrifugation at top speed for 5 minutes or incubation 60°C for 5 minutes.

2. RNA Contamination

» Prior to the use of **PDX1 Buffer**, ensure that RNase A added **PDX1 Buffer** is not out of the date. If so, add additional RNase A .

» Too many bacterial cells were used. Reduce sample volume.

3. Genomic DNA Contamination

» Do not use overgrown bacterial culture.

» During **PD2 Buffer** addition, mix gently to prevent genomic DNA from shearing.

4. Nuclease Contamination

» If the host cells have high nuclease activity (eg. *End*A + Strain), perform this optional Wash Step to remove residual nuclease activity.

a. After DNA Binding Step, add 250 µl of **WX1 buffer** into **PDX Column** and incubate for 2 minutes at room temperature.

b. Centrifuge at 7,000 x g for 30 seconds.

c. Following using standard Wash Step.

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