Ver. 2104-01

PureHelix™ *Fast-n-Pure Plasmid* Kit (Ver. 2.0)

Kit contents

PureHelix™ *Fast-n-Pure Plasmid* Kit (Ver.2.0)		
Cat. No.	**FPL50**	**FPL200**
Buffer S1	15 ml	60 ml
Buffer S2	15 ml	60 ml
Buffer EB	5 ml	20 ml
Buffer WB	15 ml **(Add 60 ml ethanol)**	40 ml **(Add 160 ml ethanol)**
RNase A	2 ea	2 ea
Column Set (without cap) 50ea/**Blue Box**	1 box	4 box
MaxBinder™ solution	5 ml	20 ml
Certificate Analysis	1 ea	1 ea

Description

PureHelix™ *Fast-n-Pure Plasmid* Kit is a simplified and fast kit for isolation of pure plasmids up to 20 μg for routine molecular biological applications, such as PCR, cloning, sequencing, in vitro transcription, transfection experiments, etc. **PureHelix™ *Fast-n-Pure Plasmid* Kit** is based on alkaline-lysis method and the binding properties of DNA on silica membrane to provide the high-quality and high-yield plasmid DNAs. The applied pH indicating system in this kit helps to visual verification of purification process and prevent handling errors. **PureHelix™ *Fast-n-Pure Plasmid* Kit** is designed that its whole process could be completed within 10 min, while other conventional products require at least 25 min to purify the plasmid using mini-columns.

Application

Molecular biological applications
Automatic fluorescent sequencing
Restriction enzymatic digestion and cloning
Transfection of robust cells

Store

Ambient temperature

NanoHelix Co., Ltd.
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Quality Control Assay

Functional analysis

PureHelix™ *Fast-n-Pure Plasmid* Kit was tested for the isolation of high- or low-copy plasmids and fosmid DNA from *E. coli* cells containing each plasmid DNA. Also, the high-copy plasmid DNA isolated with **PureHelix™ *Fast-n-Pure Plasmid* Kit** was tested in the restriction-enzymatic digestion and the sequencing analysis.

Quality authorized by Yountaek Go 

Protocol

Important things to do before starting

- Before using **Buffer WB**, add **absolute ethanol** according to the bottle label to obtain a working solution. You may use 80% ethanol, instead of Buffer WB**.**
- This kit provides two vials of RNase A powder. **Dissolve the powder of one vial using 0.5 ~ 1 ml of Buffer S2 and transfer back into the Buffer S2 bottle.** The RNase A containing Buffer S2 should be stored at 4℃.
- The activity of dissolved RNase A in Buffer S2 could be lowered after several months and a little amount of RNA could be co-purified with plasmid. **When RNAs are detected after plasmid purification, add the additional RNase A** to the Buffer S2 enhance the enzyme activities.

1. Cell Harvest and Lysis

1) Harvest the 1 ~ 3 ml of **bacterial cell culture** by centrifugation, discard the supernatants and vigorously vortex.

※ After discarding of the supernatant, the remaining 10 ~ 50 µl of media broth doesn't affect the next purification step. The vigorous vortexing will resuspend the pelleted cells in the remaining media and help the cell lysis by Buffer S1.

2) Add **300 µl of Buffer S1** and lyse the bacterial cells by inverting the tube immediately 2 ~ 3 times.

※ Buffer S1 contains a pH-indicating purple dye which does not affect the purify and yield of DNA.

2. Neutralization

1) Add **300 µl of Buffer S2 (containing RNase A)** and mix by inverting the tube immediately 4 ~ 6 times. After mixing the solution, color should be turned to bright yellow.

2) Centrifuge at **12,000 rpm for 1 min** at room temperature.

3. Super-activation of column

1) Place a **Spin Column** into a **2 ml collection tube**.
2) Add **100 µl** of **MaxBinder™ Solution** into the Spin Column.
3) Centrifuge at **12,000 rpm for 10 sec** and immediately proceed to Step 4.
   You need not discard the flow-through from the collection tube.
   ※ These steps are required for the best yield.

4. Loading

1) Apply the **supernatants** from step 2 into the activated **Spin Column** by decanting or pipetting.
2) Centrifuge at **12,000 rpm for 10 sec** and discard the flow-through.

5. Washing

1) Apply **750 µl of Buffer WB (80% ethanol)** into the **Spin Column.** Centrifuge at **12,000 rpm for 10 sec** and discard the flow-through.
   ※ Repeat this step for the high-purity DNA preparation.

2) **Centrifuge again for 2 min** to remove residual ethanol.
   ※ The Buffer WB bottle should be closed well immediately after use. The content of ethanol in the aged Buffer WB maybe decreased under 70% and this will be resulted in low yield of DNA.

6. Elution

1) Place the **Spin Column** into a **1.5 ml microtube (not provided).**
2) Add **30 ~ 50 µl of Buffer EB** or distilled water to the center of column membrane and incubate for 1 min at room temperature.
3) Centrifuge at **12,000 rpm for 1 min** to elute DNA.

Products

Cat. No.	Products	Size
FPL50	PureHelix™ *Fast-n-Pure Plasmid* Kit (Ver. 2.0)	50 preps
FPL200	PureHelix™ *Fast-n-Pure Plasmid* Kit (Ver. 2.0)	200 preps

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