USER GUIDE

HiYield™ plasmid Kit *Mini*

Cat. AHP100, AHP300
Qty. 100r, 300r

Ver. 2023 - 12

ARROWTEC

Content and Storage

Cat.	Product.	Qty.
AHP100	**HiYield™ Plasmid Kit *Mini***	100
AHP300		300

Store at room temperature 15 ℃~25 ℃

Content.	**AHP100**	**AHP300**
PH1 Buffer	25 ml	65 ml
PH2 Buffer	25 ml	75 ml
PH3 Buffer	40 ml	100 ml
W1 Buffer	50 ml	130 ml
Wash Buffer (concentrated)	25 ml *	40 ml **
Elution Buffer	10 ml	30 ml
RNase A (50mg/ml)	50 µl	130 µl
2ml Collection Tube	100 pcs	300 pcs
PH Column	100 pcs	300 pcs

Sample Source: Plasmid DNA from Bacteria **Preparation time :** 20 min
Sample Size: 1-4ml of LB broth overnight incubate bacterial cultures
Typical Plasmid Yield :
Low Copy Number : 0.5-5µg **// High Copy Number :** 10-20µg (max 30 µg)
Add provided RNase A to PH1 Buffer and store at 4°C. If precipitates have formed in PH2 Buffer, warm the buffer at a 37°C water bath to dissolve.
* Add 100ml ethanol (95-99.9%) to Wash Buffer prior to initial use.
** Add 160ml ethanol (95-99.9%) to Wash Buffer prior to initial use.

Description

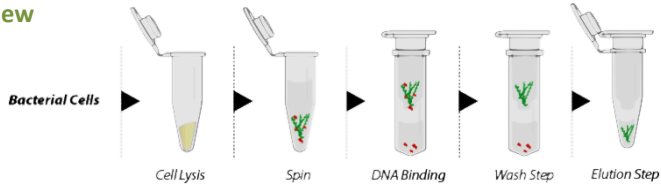
The **HiYield™ Plasmid Kit *Mini*** is designed for rapid isolation of plasmid or cosmid DNA from 1-4 ml of bacterial cultures. The modified alkaline lysis method followed by RNase treatment is utilized to obtain cleared cell lysate with minimal genomic DNA or RNA contamination. Silica spin technology coupled with chaotrophic salt provides a reliable DNA binding and elution system. Purified DNA is ready for restriction digestion, ligation, PCR and sequencing reaction.

Quality Control

The quality of **HiYield™ Plasmid Kit *Mini*** are tested on a lot-to-lot basis.
The Kits are tested by isolation of plasmid DNA from 4 ml culture of *E.coli* DH5a transformed with the plasmid pUC19 (A_{600}>2units/ml).

Standard Protocol

Overview



Cell Harvesting

1. Transfer 1.5 ml of bacterial culture to a microcentrifuge tube.
2. Centrifuge for 1 min at full speed (13,000 rpm).

Resuspension

3. Add 200 µl of ***PH1 Buffer*** (RNase A added) and resuspend the cell pellet by vortex.

Lysis

4. Add 200 µ l of ***PH2 Buffer*** and mix gently by inverting the tube 10 times. **Do Not Vortex**, avoid shearing genomic DNA.
5. Allow mixture to stand for 2 min at room temperature.

Neutralization

6. Add 300 µl of ***PH3 Buffer*** and mix immediately by inverting the tube 10 times. **Do Not Vortex**
7. Centrifuge for 2 min at full speed (13,000 rpm).

DNA Binding

8. Place at ***PH column*** in a 2ml Collection Tube.
9. Apply the clear lysate(supernatant) from Step 7 to the PH Column.
10. Centrifuge at full speed (13,000 rpm) for 30 seconds.
11. Discard the flow-through and return the ***PH Column*** back to the 2ml ***Collection Tube***.

Wash

12. Add 400 µl of ***W1 Buffer*** in the ***PH column***.
13. Centrifuge at full speed (13,000 rpm) for 30 seconds.
14. Discard the flow-through and return the ***PH Column*** to the 2ml ***Collection Tube***.
15. Add 600 µl of ***Wash Buffer*** (ethanol added) to ***PH Column***.
16. Centrifuge at full speed (13,000 rpm) for 30 seconds.
17. Discard the flow-through and return the ***PH Column*** to the 2ml ***Collection Tube***.
18. Centrifuge again for 3 min at full speed to dry the column matrix.

Standard Protocol (continued)

DNA Elution

19. Transfer the dried PH Column to a clean 1.5 microcentrifuge tube.
20. Add 50 µl of Elution Buffer or ddH_2O (pH 8.0-8.5) directly onto the centre of the membrane. Avoid residual buffer adhering to the wall of the column.
21. Allow to stand for 2 min until the liquid is absorbed.
22. Centrifuge for 2 min at full speed (13,000 rpm) to elute plasmid DNA.

Low Copy Number Protocol

Add ethanol and RNase A to buffer according to component instructions.
The typical yield is about 0.5-1.0 µg per 1ml culture when preparing low-copy-number plasmid from overnight bacterial culture in LB or TB medium. If the plasmid is larger than 30kb, preheat the Elution Buffer to 70℃ prior to the Elution Step.

Cell Harvesting

1. Harvest up to 10ml of overnight culture by centrifugation.

Resuspension

2. Add 400 µl of ***PH1 Buffer*** (RNase A added) and resuspend the cell pellet by vortex.

Lysis

3. Add 400 µ l of ***PH2 Buffer*** and mix gently by inverting the tube 10 times. **Do Not Vortex**, avoid shearing genomic DNA.
4. Allow mixture to stand for 2 min at room temperature.

Neutralization

5. Add 600 µl of ***PH3 Buffer*** and mix immediately by inverting the tube 10 times. **Do Not Vortex**
6. Centrifuge for 3 min at full speed (13,000 rpm).

DNA Binding

7. Place at ***PH column*** in a 2ml ***Collection Tube***.
8. Apply 750 µl of the clear lysate (supernatant) from Step 6 to the ***PH Column***.
9. Centrifuge at full speed (13,000 rpm) for 30 seconds. Discard the flow-through and return the ***PH column*** to the 2ml ***Collection Tube***.
10. Apply the remaining clear lysate to the same ***PH column***.
11. Centrifuge at full speed (13,000 rpm) for 30 seconds.
12. Discard the flow-through and return the ***PH Column*** back to the 2ml ***Collection Tube.***

Low Copy Number Protocol (continued)

Wash

13. Add 400 µl of ***W1 Buffer*** in the ***PH column***.
14. Centrifuge at full speed (13,000 rpm) for 30 seconds.
15. Discard the flow-through and return the ***PH Column*** to the 2ml ***Collection Tube***.
16. Add 600 µl of ***Wash Buffer*** (ethanol added) to ***PH Column***.
17. Centrifuge at full speed (13,000 rpm) for 30 seconds.
18. Discard the flow-through and return the ***PH Column*** to the 2ml ***Collection Tube***.
19. Centrifuge again for 3 min at full speed to dry the column matrix.

20. Transfer the dried ***PH Column*** to a clean 1.5 microcentrifuge tube.
21. Add 50 µl of Elution Buffer or ddH_2O (pH 8.0-8.5) to the center of the column matrix.
    If plasmid DNA is larger than 10kb, use preheated Elution Buffer (70℃) during Elution Step to improve the elution efficiency.
22. Allow to stand for 2 min until the liquid is absorbed.
23. Centrifuge for 2 min at full speed (13,000 rpm) to elute plasmid DNA.

Troubleshooting

Problem	Possible Reason/Solution
Low yield	***Bacterial cells were not lysed completely*** Too many bacterial cells were used. If using more than 10 A_{600} units of bacterial culture, separate into multiple tubes. Following PH3 Buffer addition, break up the precipitate by inverting to ensure higher yield.
	Incorrect Wash Buffer Check to ensure Ethanol was added to Wash Buffer prior to use.
	Incorrect DNA Elution Step Ensure that Elution Buffer was added and absorbed to the center of PH Column matrix.
	Incomplete DNA Elution If plasmid DNA is larger than 10kb, use preheated Elution Buffer (70°C) during Elution Step to improve the elution efficiency.

Troubleshooting

Problem	Possible Reason/Solution
Eluted DNA does not perform well in downstream applications	***Residual ethanol contamination*** After wash step, dry PH Column with additional centrifugation at top speed for 5minutes or incubation at 60°C for 5minutes.
	RNA Contamination Prior to using PH1 Buffer, ensure that RNase A was added. If RNase A added PH1 Buffer is out of date, add additional RNase A. Too many bacterial cells were used, reduce sample volume.
	Genomic DNA contamination Do not use overgrown bacterial culture. During PH2 and PH3 Buffer addition, mix gently to prevent genomic DNA shearing.
	Nuclease contamination If host cells have high nuclease activity(e.g.,endA$^+$ strains), perform this Optional Wash Step to remove residual nuclease. After DNA Binding Step, add 200 µl of PH3 Buffer to PH Column and incubate for 2 min at room temperature. Centrifuge at 6000 xg (8,000rpm) for 30 seconds. Continue from standard Wash Step.