# Quick Protocol

## PlasJia Mini Plasmid Extraction Kit

DNA isolation based on silica technology

- MiniPrep

## For DNA Isolation from

Bacterial Cultured Cell

## Kit Content

| Component | 50 preps |
|---|---|
| **SOB** | 12.5 ml |
| **PLB** | 12.5 ml |
| **NOT** | 17.5 ml |
| **PWB (Concentrated)** | 12.5 ml |
| **PEB** | 5 ml |
| **Prime RNase A** | 1.5 mg |
| **HiPure DR Column Column** | 50 |
| **Collection tube** | 100 |

## Recommended Starting Material

### Growth of Bacterial Cultures

- Pick a single colony from a freshly streaked selective plate to inoculate 1-5 ml of LB medium supplemented with the appropriate selection antibiotic. Incubate for 12-16 hours at 37°C while shaking at 200-250 rpm. Use a tube or flask with a volume of at least 4 times the culture volume.
- Harvest the bacterial culture by centrifugation at 13,000 rpm in a microcentrifuge for 3 min at room temperature. Decant the supernatant and remove all remaining medium.

### Do not overload the column

- For high-copy-number plasmids, do not process more than 5 ml of bacterial culture. If more than 5 ml of such a culture are processed, the spin column capacity (20 µg of dsDNA) will be exceeded and no increase in plasmid yield will be obtained.
- For low-copy-number plasmids, it may be necessary to process larger volumes of bacterial culture (up to 10 ml) to recover a sufficient quantity of DNA.

## Before Start

- All centrifugation steps are carried out at room temperature (15–25°C).
- Do not forget to add the appropriate amount of ethanol (96%–100) to PWB as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- Check the Lysis Solution (PLB) and the Neutralization Solution (NOT) for salt precipitation before each use. Redissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use. Do not shake the Lysis Solution too vigorously.
- Both the Lysis Solution (PLB) and the Neutralization Solution (NOT) contain irritants. Wear gloves when handling these solutions.
- Add RNase A (1.5 mg) to SOB, mix and store at 4°C. After addition of RNase A, the Resuspension Solution (SOB) can be used for 6 months when stored at 4°C.

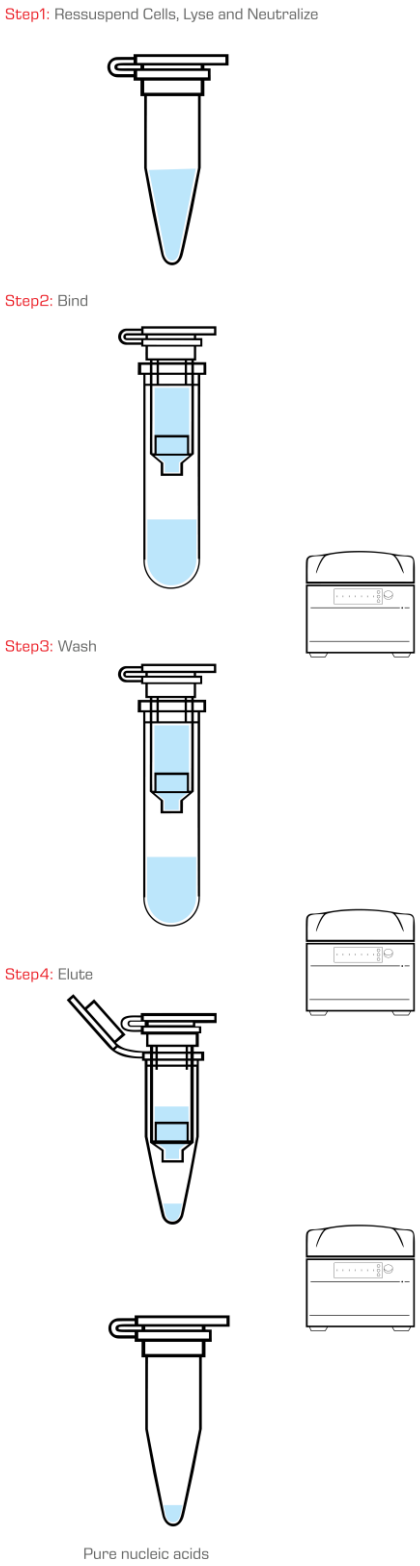
## Buffer Preparation

- Add Prime RNase A (1.5mg) to SOB, mix and store at 4°C. After addition of RNase A, the Resuspension Solution (SOB) can be used for 6 months when stored at 4°C.
- Add 12.5ml ethanol (96-100%) to PWB before use.

**Table 1:** Washing buffer preparation

| Buffer Name | Concentrated Volume | Amount of Ethanol | Final Volume |
|---|---|---|---|
| PWB | 12.5 | 12.5 | 25 |

# Procedure of silica-based DNA isolation in quick look



# Protocol

## Protocol: Isolation of Plasmid DNA

### Sample type:

- Bacteria Cultured Cell

### Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Do not forget to add the appropriate amount of ethanol (96%–100) to PWB as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- Check the Lysis Solution (PLB) and the Neutralization Solution (NOT) for salt precipitation before each use. Redissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use. Do not shake the Lysis Solution too vigorously.
- Both the Lysis Solution (PLB) and the Neutralization Solution (NOT) contain irritants. Wear gloves when handling these solutions.
- Add Prime RNase A (1.5 mg) to SOB, mix and store at 4°C. After addition of RNase A, the Resuspension Solution (SOB) can be used for 6 months when stored at 4°C.

## Process

1- Growth of bacterial cultures in tube or falcon and harvesting: Harvest the bacterial cells from 1 ~ 5ml recombinant E. *coli* culture by centrifugation of 13,000 rpm in a conventional, table-top micro-centrifuge for 3 min at room temperature.
2- Add 250 µl of SOB to the collected cells and completely re-suspend by vortexing or pipetting.
3- Add 250 µl of PLB and mix by inverting the tube 3~5 times by inverting, gently and incubate for 1min: Vortexing may cause shearing of genomic DNA. Do not vortex.
4- Add 350 µl of NOT and immediately mix by inverting the tube 3~5 times, gently: Genomic DNA and cell debris will be formed and insoluble complex. Do not vortex.
5- Centrifugation the tube at 13,000 rpm, 4 ~ 25°C for 10 min in micro-centrifuge: A compact white pellet will be appeared at the bottom of the tube.
6- Transfer the supernatant (cleared lysate) to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
7- Add 500 µl of Washing Buffer (PWB) to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.
8- Dry the spin column by additional centrifugation at 13,000 rpm for 3 min to remove the residual ethanol in spin column.
9- Transfer the spin column to the new 1.5 ml micro-centrifuge tube (Not provided).
10- Add 30 ~ 100 µl of PEB or Nuclease free water to the spin column with micro-centrifuge tube, and let stand for at least 1 min.
11- Elute the plasmid DNA by centrifugation at 13,000 rpm for 1 min.
12- Discard the column and store the purified plasmid DNA at -20°C.