

## Plasmid Extraction Kit

(Mini Prep)

Cat. No: EX6112

Store kit at: RT

Store RNase A at: -20 °C

Quantity: 50 preps

### Kit Contents

RB Buffer	12.5ml
LB Buffer	12.5ml
NB Buffer	17.5ml
WB Buffer (concentrate) *	7ml
EB Buffer **	5ml
RNase A	125µl
Mini Column	50
Protocol Handbook	1

\* Before first use, add absolute ethanol (ACS grade or better) into Buffer WB as indicated on the bottle (add 28ml absolute ethanol in WB Buffer before use)

\*\* 10 mM TrisCl, pH 8.5

### Storage conditions

SinaClon Plasmid extraction Kit is shipped at room temperature. All components are stable at room temperature until the date of expiration that is printed on the product label. After addition of RNase A, buffer RB is stable for 1 year when stored at 4°C. In cold ambient condition, Buffer LB and NB may exhibit salt precipitation and this will cause reduction of DNA recover-yields. If so, heat the bottle with occasional swirling in 37 °C water bath until completely dissolved.

### Safety Information

SinaClon Plasmid extraction Kit contain the irritant which is harmful when in contact with skin, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

### Introduction

SinaClon Plasmid extraction Kit provides easy and rapid method for the small-scale preparation of plasmid DNA from bacterial cells. This kit can be used to isolate and purify any plasmid, but works most efficiently when the plasmid is less than 20 kb in size, all process to prepare pure plasmid DNA takes only about 45 min and simultaneous processing of multiple samples can be easily performed. Up to 2-5 µg of pure plasmid can be purified using SinaClon Plasmid extraction mini kit and this pure plasmid DNA is ready for PCR, cloning, fluorescent sequencing, synthesis of labeled hybridization probes, cell transfection, electroporation, and enzymatic restriction analysis without further manipulation.

### Starting material

The yield and quality of plasmid DNA depends on several factors such as plasmid copy number, bacterial strain, antibiotics, inoculation and type of culture medium. Wherever possible, plasmids should be purified from bacterial cultures that have been inoculated with a single transformed colony picked from an agar plate. Usually, the colony is

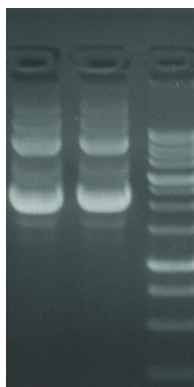
transferred to a small starter culture, which is grown to late log phase. Aliquots of this culture can be used to prepare small amounts of the plasmid DNA for analysis and/or as the inoculum for a large-scale culture. The conditions of growth of the large-scale culture depend chiefly on the copy number of the plasmid and whether it replicates in a stringent or relaxed fashion. At all times, the transformed bacteria should be grown in selective conditions, i.e., in the presence of the appropriate antibiotics.

This kit is optimized to Luria-Bertani (LB) broth which is the most widely used culture medium for propagation of *E.coli*. Use of other rich broth such as Terrific Broth (TB) or 2XYT will lead to very high cell density. If these media are used, starting sample volume should be reduced not to overload Plasmid column and buffer system. Otherwise, the volume of buffer RB, LB and NB should be increased for efficient lysis. Overnight culture in TB or 2XYT may yield 2 - 5 times the number of cells compared to cultures grown in LB broth. TB or 2XYT can be used to obtain more yield of plasmid DNA, in case of low-copy number plasmid.

### **Elution**

Purified DNA can be eluted in low salt buffer or deionized water as need for downstream applications. Buffer EB contains 10 mM TrisCl, pH8.5. When using water as eluent, make sure that the pH value is within 7.0 and 8.5. Because plasmid in water is susceptible to hydrolysis and lacks a buffering agent, it is recommended to store below -20 °C. For higher concentration of DNA, decrease the volume of elution buffer, for higher yield, increase the volume of elution buffer and repeat the elution step once or twice again based on copy number of plasmid.

<b>OD: 260/280</b>	<b>OD: 260/230</b>	<b>Yield</b>
<b>1.88</b>	<b>2.13</b>	<b>2-5µg</b>



**Figure 1.** Plasmid DNA was extracted using **SinaClon Plasmid extraction Kit**

### **Before Experiment**

\*Unless there is an another indication, all centrifugation steps should be performed at full speed (> 1 0,000 g or 10,000 ~ 14,000 rpm)

\* **Add all of RNase solution to RB Buffer before first use.**

\* **Store the RB buffer at 4°C after addition of RNase.**

\* Prepare new 1.5 ml or 2 ml tubes.

\* LB and NB Buffer may precipitate at cool ambient conditions. If precipitate appears, dissolve it in 37 °C water bath until completely dissolved.

### Preparation

- 1. Pellet the bacterial culture by centrifugation for 5 min at 13,000 rpm in a tabletop centrifuge. Discard the supernatant as much as possible.**

Use appropriate volume of bacterial cultures; up to 5 ml for high copy number plasmid, or up to 10 ml for low copy number plasmid. Bacterial culture should be grown for (16 to 21) hours in LB media containing a selective antibiotics. Use of other rich broth, such as TB or 2XYT, and/or higher culture volume can cause reduction of lysis efficiency or overload of a mini column, resulting in unsatisfactory yields.

Alternatively, bacterial cells can be pelleted repeatedly in 1.5 ml or 2 ml microcentrifuge tube, by centrifugation for 1 min at full speed.

- 2. Resuspend pelleted bacterial cells thoroughly in 250 µl of RB Buffer. Transfer the suspension to a new 1.5 ml tube.**

It is essential to thoroughly resuspend the cell pellet and then place at for 15 minutes at 37°C.

You don't need to transfer the suspension if the cells have been pelleted in a 1.5 ml tube at previous step.

\*Add RNase solution to RB buffer before first use.

- 3. Add 250 µl of LB Buffer and mix by inverting the tube 4 times (DO NOT VORTEX).**

Incubate until the cell suspension becomes clear and viscous, but DO NOT incubate for more than 5 min. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps.

If precipitated material has formed in LB Buffer before use, heat to dissolve at 37 °C, Precipitated LB Buffer may cause significant decrease in DNA recover yield.

- 4. Add 350 µl of NB Buffer and immediately mix by inverting the tube 4-6 times (DO NOT VORTEX).**

For better precipitation, mix the lysate gently but completely and immediately after addition of NB Buffer.

Precipitation is enhanced by using chilled NB Buffer and incubating on ice around 10 min. After addition of NB Buffer, a fluffy white material forms and the lysate becomes less viscous.

- 5. Centrifuge for 10 min.**

For better precipitation of the debris do centrifugation step in 4°C.

- 6. Transfer carefully the supernatant to a mini Column by decanting or pipetting. Centrifuge for 2min. Remove the mini Column, discard the pass-through, and re-insert the mini Column to the collection tube.**

Avoid the white precipitate cotransferring into the mini column.

- 7. Apply 700 µl of WB Buffer and centrifuge for 1min. Remove the mini Column, discard the pass-through, and re-insert the mini Column to the collection tube.**

If the purified DNA will be used for salt sensitive applications, let the mini column stand for 5 min after addition of WB Buffer, making some amount of wash buffer flow through the column by gravity before centrifugation.

- 8. Centrifuge for an additional 1 min to remove residual wash buffer. Transfer the mini Column to a new 1.5 ml tube (Not provided).**

This step removes residual ethanol from mini column membrane. Residual Ethanol in elute may inhibit subsequent enzymatic reaction. If carry over of WB Buffer occurs, centrifuge again for 2 min before proceeding to next step.

**9. Add 50 µl of EB Buffer or deionized distilled water, let stand for 5-10 min, and centrifuge for 1 min.**

Ensure that EB buffer or distilled water is dispensed directly onto the center of spin column membrane for optimal elution of DNA.

Some larger plasmids (> 10 kb) usually may not be eluted optimally unless preheated (70°C) buffer EB or ddH<sub>2</sub>O is applied for elution. Incubate for 2 min after addition of pre-heated elution buffer.

### Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no yield of plasmid DNA	<p>Too many cells in sample</p> <p>Low-copy-number plasmid used</p> <p>Poor resuspension of bacterial pellets in Buffer RB</p> <p>Buffer LB precipitate</p> <p>Insufficient digestion with RNase</p>	<p>Cultures should be grown for 16 — 21 hours in proper media with antibiotics. Reduce the volume of sample. If rich broth such as Terrific Broth (TB) or 2XYT is used, starting sample volume must be reduced because these media have very high cell density (2 - 5 times to LB)</p> <p>Low-copy-number plasmid may yield as little as 0.5 µg of DNA from a 5 ml overnight culture. Increase the culture volume or use high-copy-number plasmid or rich broth, if possible.</p> <p>Bacterial cell pellets must be thoroughly resuspended in Buffer RB.</p> <p>Redissolve Buffer LB by warming at 37°C (or above).</p> <p>Excess RNA can interfere the binding of plasmid DNA with Plasmid mini column membrane. Store buffer RB at 4°C after the addition of RNase. If buffer RB containing RNase is more than a year old, the activity of RNase can be decreased slightly.</p>
Chromosomal DNA contamination	Mis-handling of the lysate after addition of Buffer NB	<p>igorous vortexing after addition of Buffer NB can cause shearing of chromosomal DNA followed by chromosomal DNA contamination.</p> <p>Handle gently the lysate after addition of Buffer NB. Simple inverting and rotating tube to cover walls with lysate is sufficient for mixing.</p>
Smearing of plasmid DNA	<p>Too long lysis time</p> <p>Vigorous mixing in Buffer LB</p>	<p>Too long lysis under Buffer LB can cause chromosomal DNA contamination. Proceed to next step immediately after no more clumps are visible in the lysate.</p> <p>Lysis time should not be over 5 min in any Case.</p> <p>Vigorous handling after addition of buffer LB can lead to irreversible denaturation of plasmid DNA. Gentle inverting and rotating tube to cover walls with viscous lysate is sufficient for mixing.</p>
RNA Contamination	<p>RNase omitted or old</p> <p>Too many cells in sample</p>	<p>RNase solution should be added to buffer RB before first use. If buffer RB containing RNase is more than a year old, the activity of RNase can be decreased. Add additional RNase (working concentration = 100 µg/ml). Buffer RB containing RNase should be stored at 4°C.</p> <p>Reduce the sample volume. Too many cells may not be subjected properly to RNase digestion.</p>
DNA floats out of well while loading of agarose gel	Ethanol is not completely removed during wash step	Ensure that washing steps are performed properly.