# Plasmid Extraction Kit

**(Mini Prep)**

**Cat. No: EX٦١١٢** **Store kit at: RT**

**Store RNase A at: -٢٠ °C** **Quantity: ٥٠ preps**

## Kit Contents

| | |
|---|---|
| RB Buffer | ١٢,٥ml |
| LB Buffer | ١٢,٥ml |
| NB Buffer | ١٧,٥ml |
| WB Buffer (concentrate) * | ٧ml |
| EB Buffer ** | ٥ml |
| RNase A | ١٢٥µl |
| Mini Column | ٥٠ |
| Protocol Handbook | ١ |

* Before first use, add absolute ethanol (ACS grade or better) into Buffer WB as indicated on the bottle (add ٢٨ml absolute ethanol in WB Buffer before use)

## 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 I year when stored at ٤°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 ٣٧ °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 ٢٠ kb in size, all process to prepare pure plasmid DNA takes only about ٤٥ min and simultaneous processing of multiple samples can be easily performed. Up to ٢-٥ 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 ۲XYT 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 ۲XYT may yield ۲ - ۵ times the number of cells compared to cultures grown in LB broth. TB or ۲XYT 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 ۱۰ mM TrisCI, pH۸,۵. When using water as eluent, make sure that the pH value is within ۷,۰ and ۸,۵. Because plasmid in water is susceptible to hydrolysis and lacks a buffering agent, it is recommended to store below -۲۰ °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.

| OD: ۲۶۰/۲۸۰ | OD: ۲۶۰/۲۳۰ | Yield |
|---|---|---|
| ۱,۸۸ | ۲,۱۳ | ۲-۵µg |

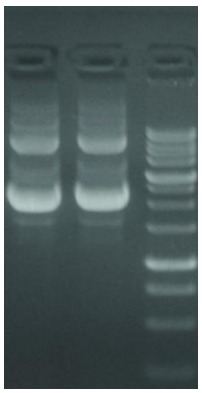

**Figure ۱.** Plasmid DNA was extracted using **SinaClon Plasmid extraction Kit**

**Before Experiment**

*Unless there is an another indication, all centrifugation steps should be performed at full speed (> ١٠,٠٠٠ g or ١٠,٠٠٠ ~ ١٤,٠٠٠ rpm)

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

*** Store the RB buffer at ٤°C after addition of RNase.**

* Prepare new ١,٥ ml or ٢ ml tubes.

* LB and NB Buffer may precipitate at cool ambient conditions. If precipitate appears, dissolve it in ٣٧ °C water bath until completely dissolved.

**Preparation**

١. **Pellet the bacterial culture by centrifugation for ٥ min at ١٣,٠٠٠ rpm in a tabletop centrifuge. Discard the supernatant as much as possible.**
Use appropriate volume of bacterial cultures; up to ٥ ml for high copy number plasmid, or up to ١٠ ml for low copy number plasmid. Bacterial culture should be grown for (١٦ to ٢١) hours in LB media containing a selective antibiotics. Use of other rich broth, such as TB or ٢XYT, 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 ١,٥ ml or ٢ ml microcentrifuge tube, by centrifugation for I min at full speed.

٢. **Resuspend pelleted bacterial cells thoroughly in ٢٥٠ µl of RB Buffer. Transfer the suspension to a new ١,٥ ml tube.**
It is essential to thoroughly resuspend the cell pellet and then place at for ١٥ minutes at ٣٧°C.
You don't need to transfer the suspension if the cells have been pelleted in a ١,٥ ml tube at previous step.
*Add RNase solution to RB buffer before first use.

٣. **Add ٢٥٠ µl of LB Buffer and mix by inverting the tube ٤ times (DO NOT VORTEX).**
Incubate until the cell suspension becomes clear and viscous, but DO NOT incubate for more than ٥ 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 ٣٧ °C, Precipitated LB Buffer may cause significant decrease in DNA recover yield.

٤. **Add ٣٥٠ µl of NB Buffer and immediately mix by inverting the tube ٤-٦ 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 ١٠ min. After addition of NB Buffer, a fluffy white material forms and the lysate becomes less viscous.

٥. **Centrifuge for ١٠ min.**
For better precipitation of the debris do centrifugation step in ٤°C.

٦. **Transfer carefully the supernatant to a mini Column by decanting or pipetting. Centrifuge for ٢min. Remove the mini Column, discard the pass-through, and re-insert the mini Column to the collection tube.**
Avoid the white precipitate cotransfering into the mini column**.**

٧. **Apply ٧٠٠ µl of WB Buffer and centrifuge for ١min. 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 ٥ min after addition of WB Buffer, making some amount of wash buffer flow through the column by gravity before centrifugation.

٨. **Centrifuge for an additional ١ min to remove residual wash buffer. Transfer the mini Column to a new ١,٥ ml tube (Not provided).**
This step removes residual ethanol from mini column membrane. Residual Ethanol in elute may inhibit subsequent enzymatic reaction. If carryover of WB Buffer occurs, centrifuge again for ٢ min before proceeding to next step.

٩. **Add ٥٠ µl of EB Buffer or deionized distilled water, let stand for ٥-١٠ min, and centrifuge for ١ 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 (> ١٠ kb) usually may not be eluted optimally unless preheated (٧٠°C) buffer EB or ddH٢O is applied for elution. Incubate for ٢ min after addition of pre-heated elution buffer.

## Troubleshooting Guide

| Facts | Possible Causes | Suggestions |
|---|---|---|
| Low or no yield of plasmid DNA | Too many cells in sample | Cultures should be grown for ١٦ — ٢١ hours in proper media with antibiotics. Reduce the volume of sample, If rich broth such as Terrific Broth (TB) or ٢XYT is used, starting sample volume must be reduced because these media have very high cell density (٢ - ٥ times to LB) |
| | Low-copy-number plasmid used | Low-copy-number plasmid may yield as little as ٠,٥ ug of DNA from a ٥ ml overnight culture. Increase the culture volume or use high-copy-number plasmid or rich broth, if possible. |
| | Poor resuspension of bacterial pellets in Buffer RB | Bacterial cell pellets must be thoroughly resuspended in Buffer RB. |
| | Buffer LB precipitate | Redissolve Buffer LB by warming at ٣٧°C (or above). |
| | Insufficient digestion with RNase | Excess RNA can interfere the binding of plasmid DNA with Plasmid mini column membrane. Store buffer RB at ٤°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. |
| Chromosomal DNA contamination | Mis-handling of the lysate after addition of Buffer NB | igorous vortexing after addition of Buffer NB can cause shearing of chromosomal DNA followed by chromosomal DNA contamination. Handle gently the lysate after addition of Buffer NB. Simple inverting and rotating tube to cover walls with lysate is sufficient for mixing. |
| Smearing of plasmid DNA | Too long lysis time | 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. Lysis time should not be over ٥ min in any Case. |
| | Vigorous mixing in Buffer LB | 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. |
| RNA Contamination | RNase omitted or old | 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 = ١٠٠ ug/ ml). Buffer RB containing RNase should be stored at ٤°C. |

| | | |
|---|---|---|
| | Too many cells in sample | Reduce the sample volume. Too many cells may not be subjected properly to RNase digestion. |
| 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. |