SinaClon
شرکت سیناکلون BioScience

Plasmid Extraction Kit

(Mini Prep)

REF EX6112 Σ 50 TESTS

RUO

-20 °C Store RNase A

15°C 25°C Store kit

Components

Contents	Quantity/ Volume	Store Temperature
RB Buffer	12.5ml	RT
LB Buffer	12.5ml	RT
NB Buffer	17.5ml	RT
WB Buffer(concentrate)	7ml	RT
EB Buffer	5ml	RT
RNase A	125µl	-20
Mini Column	50	RT
Protocol Handbook	1	RT

Description

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 20kb in size, all process to prepare pure plasmid DNA takes only about 45min and simultaneous processing of multiple samples can be easily performed. Up to 2-5µg 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.

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

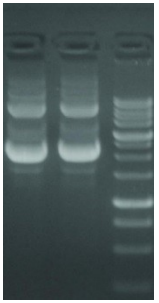

Figure 1. Plasmid DNA was extracted using SinaClon Plasmid extraction Kit

Important notes: please read before starting

- 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).
- 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.
- **To increase the stability of RNase A, Keep Enzyme Separately at -20℃ and use it per reaction as it is written in the protocol but It is suggested that to reduce the pipetting error, add total amount of RNase A into the RB Buffer before first use, mix thoroughly, then keep the total buffer at 4℃ and use as soon as possible.**
- Precipitation is enhanced by using chilled NB Buffer. keep NB Buffer on ice or at refrigerator before using.
- 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.

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.

Preparation

1. Pellet the bacterial culture by centrifugation for 5min at 13,000rpm. Discard the supernatant as much as possible.

Use appropriate volume of bacterial cultures; up to 5ml for high copy number plasmid, or up to 10ml for low copy number plasmid. Bacterial culture should be grown for (16 to 21) hours in LB media containing a selective antibiotic.

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

2. Resuspend pelleted bacterial cells thoroughly in 250µl of RB Buffer and then transfer the suspension to a new 1.5ml tube.

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

3. add 2.5µl RNase A and incubate at 37°C for 15min.

4. 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 5min. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps.

5. Add 350µl of NB Buffer, mix immediately by inverting the tube 4-6 times (DO NOT VORTEX) and incubate on ice around 10min.

After addition of NB Buffer, a fluffy white material forms and the lysate becomes less viscous.

6. Centrifuge for 10 min.

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

7. 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 while transferring into the mini column.

8. Apply 700µl 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 5min after addition of WB Buffer, making some amount of wash buffer flow through the column by gravity before centrifugation.

9. Centrifuge for an additional 1min to remove residual wash buffer. Transfer the mini Column to a new 1.5ml 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 2 min before proceeding to next step.

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

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 (> 10kb) usually may not be eluted optimally unless preheated (70°C) buffer EB or ddH2O is applied for elution. Incubate for 2min after addition of pre-heated elution buffer.

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 plasmids.

Signs

Signs	Definitions
	Temperature range on product use
RUO	For Research Use Only
	Name and address of the manufacturer of the product
REF	Product technical code

Troubleshooting

This guide may help to solve problems that may arise.

Facts	Possible Causes	Suggestions
Low or no yield of plasmid DNA	Too many cells in sample	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)
	Low-copy-number plasmid used	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 37°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 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.
Chromosomal DNA contamination	Mis-handling of the lysate after addition of Buffer NB	vigorous 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 5 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	Keep Enzyme at -20˚C. RNase A added to total RB Buffer, should be stored at 4°C. If buffer RB containing RNase is more than a year old, the activity of RNase can be decreased. Add additional RNase (working concentration = 100 ug/ml).
	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.

SinaClon BioScience شرکت سیناکلون

Unit 9, Rouyesh building, Science and Technology Park, Tarbiat Modares University, Pajouhesh Blvd, Tehran, Iran

+98219108211

hi@sinaclon.com

www.sinaclon.com