SLS Research Pvt. Ltd.

Plasmid DNA Purification Kit

For small-scale (mini) preparations of Plasmid DNA

User Manual

Storage

SLS' Plasmid DNA purification kit (mini) should be stored dry at room temperature (15–25°C). Kits can be stored for up to 12 months without showing any reduction in performance and quality. For longer storage these kits can be kept at 2–8°C. If any precipitate forms in the buffers after storage at 2–8°C it should be redissolved by warming the buffers to 37°C before use.

After addition of RNase A, Buffer P1 is stable for 6 months when stored at 2–8°C.

Intended Use

This Plasmid DNA purification kit (mini) is intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease. All due care and attention should be exercised in the handling of the products.

Safety Information

The buffers and the reagents may contain irritants, So when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffers PN3 and PW1 contain high concentration of chaotropic salts, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Assuarance

Each lot of the **SLS**' Plasmid DNA purification kit (mini) is tested against predetermined specifications to ensure consistent product quality.

Technical Support

At , **SLS** customers are our priority. We will assist you to overcome problems in general product usage as well as customize products for special applications. We will also develop special new products and systems especially to satisfy your needs.

We welcome your feedback to improve our products.

Kit Contents:

Contents	Quantity		Storage Temperature (upon receipt)
	25 Prep.	50 Prep.	
Spin Columns with collection tube	25	50	15-25°C
Buffer P1	10 ml	20 ml	15-25°C
Buffer P2	10 ml	20 ml	15-25°C
Buffer PN3	20 ml	25 ml	15-25°C
Buffer PW1	25 ml	30 ml	15-25°C
Buffer PW2§ (Concentrate)	4 ml	6 ml	15-25°C
Buffer PE	15 ml	15 ml	15-25°C

§ Supplied as a concentrate. See the bottle label for the preparation of working solution.

Important:

In case of any precipitation observed in the buffers, re-dissolve by warming to 37°C for some time and cool it down to room temperature before use.

Precipitation formation and re-dissolving will not harm the quality of the DNA to be extracted.

Introduction

SLS' Plasmid DNA purification kit(mini) provide a fast, simple and user friendly methods for purification of Plasmid DNA routine molecular biology laboratory applications.

This simple protocol which are ideal for simultaneous processing of multiple samples, yield pure DNA ready for direct amplification in few minutes. This protocol requires no phenol/chloroform extraction or alcohol precipitation, and involves very little handling. Plasmid DNA is eluted in Buffer PE, ready for direct application to various downstream processes. Alternatively, it can be safely stored at –20°C for later use.

The Kit is designed for quick processing of 1–24 samples simultaneously in less than 30 minutes.

Principle

SLS' plasmid purification protocol is based on a modified alkaline lysis procedure of Birnboim and Doly (1), followed by binding of plasmid DNA to silica membrane under specific salt and pH conditions.

The procedure consists of three basic steps:

1. Lysis of cell
2. Adsorption of Plasmid DNA
3. Washing and elution of plasmid DNA

All steps are performed without the use of phenol, chloroform, and without alcohol precipitation.

Application

Plasmid DNA prepared using the **SLS**' plasmid purification protocol is suitable for a variety of routine applications including:

- ✓ Restriction enzyme digestion
- ✓ Library screening
- ✓ In vitro translation
- ✓ Sequencing
- ✓ Ligation and transformation
- ✓ Transfection of robust cells

Results

Yield:

Plasmid yield with the **SLS**' plasmid purification protocol varies depending on plasmid copy number per cell, the individual insert in a plasmid, factors that affect growth of the bacterial culture, the elution volume, and the elution incubation time.

To obtain the optimum combination of DNA quality, yield, and concentration, it is recommended to use Luria Bertani (LB) medium for growth of cultures, eluting plasmid DNA in a volume of 50 µl, and performing a short incubation after addition of the elution buffer.

Benefits

- Saves time,
- Improves reproducibility and reliability,
- Minimizes DNA loss
- Ideal for a wide variety of research projects

Additional materials needed (not provided in kit)

- Absolute ethanol (96-100%)
- Standard tabletop microcentrifuge capable of a 10,000 x *g* centrifugal force.
- Microcentrifuge tubes, 1.5 ml, sterile

Important Notes before starting:

Please read the following notes before starting the procedures.

Growth of bacterial cultures in tubes or flasks

1. Pick a single colony from a freshly streaked selective plate and inoculate a culture of 1–5 ml LB medium containing the appropriate selective antibiotic. Incubate for 14–16 h at 37°C with vigorous shaking.

Growth for more than 16 h is not recommended since cells begin to lyse and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times the volume of the culture.

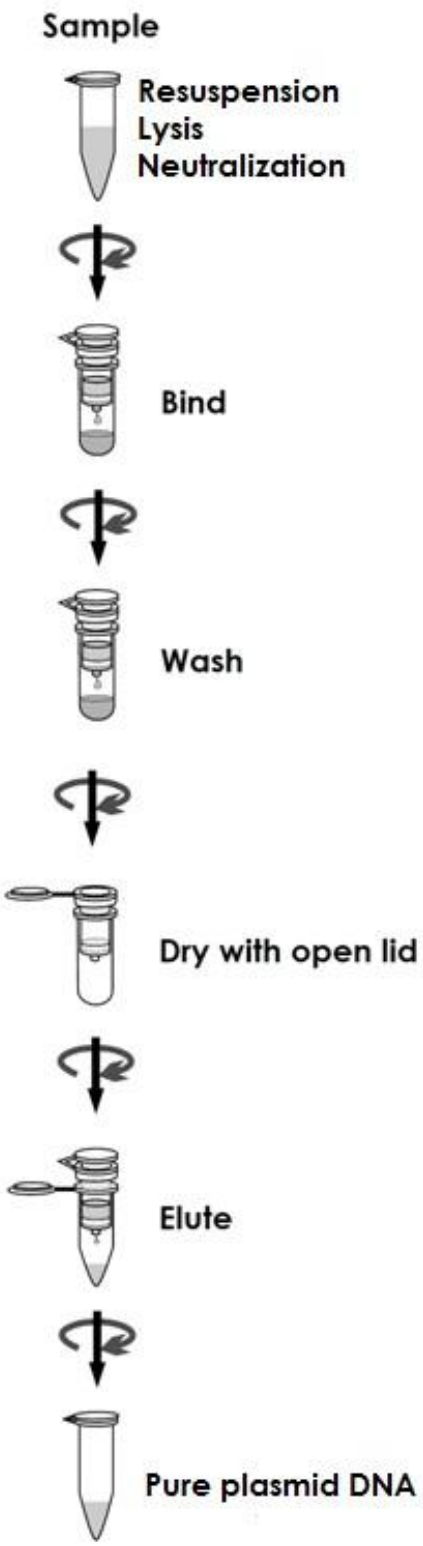
2. Harvest the bacterial cells by centrifugation at > 8000 rpm (6800 x g) in a conventional, table-top microcentrifuge for 3 min at room temperature (15–25°C).

The bacterial cells can also be harvested in 15 ml centrifuge tubes at 5400 x g for 10 min at 4°C.

For Buffers

- Add ethanol (96–100%) to Buffer PW2 before use (see bottle label for volume).
- Check Buffers P2 and PN3 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer P2 vigorously.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO_2 in the air.

SLS' Plasmid Purification Procedure



Plasmid DNA Purification

Important points to be considered

Preparation and clearing of bacterial lysate

The **SLS**' plasmid purification procedure uses the modified alkaline lysis method of Birnboim and Doly (1). Bacteria are lysed under alkaline conditions, and the lysate is subsequently neutralized and adjusted to high-salt binding conditions in one step. After lysate clearing, the sample is ready for purification by silica membrane.

DNA adsorption to the membrane

SLS' columns use a silica membrane for adsorption of plasmid DNA in high-salt buffer and elution in low-salt buffer. The optimized buffers in the lysis procedure, combined with the unique silica membrane, ensure that only DNA will be adsorbed, while RNA, cellular proteins, and metabolites are not retained on the membrane but are found in the flow-through.

Washing and elution of plasmid DNA

Endonucleases are efficiently removed by a brief wash step with Buffer PW1. The wash step is also necessary when purifying low-copy plasmids, where large culture volumes are used. Salts are efficiently removed by a brief wash step with Buffer PW2. High-quality plasmid DNA is then eluted from the column with 50–100 µl of Buffer PE or water. The purified DNA is ready for immediate use in a range of applications without precipitate, concentrate, or desalting.

Elution

- Ensure that the elution buffer is dispensed directly onto the center of the membrane for optimal elution of DNA.
- For increased DNA yield, use a higher elution-buffer volume. For increased DNA concentration, use a lower elution-buffer volume.
- If water is used for elution, make sure that its pH is between 7.0 and 8.5. Elution efficiency is dependent on pH and the maximum elution efficiency is achieved within this range. A pH <7.0 can decrease yield.

Note: Store DNA at –20°C when eluted with water, as DNA may degrade in the absence of a buffering agent.

Protocol:

IMPORTANT: Strict adherence to the assay procedure will ensure optimal assay performance. Any procedural deviation may lead to aberrant result.

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of E. coli in LB medium.

Please read "Important Notes" on pages 8 &10 before starting.
Note: All protocol steps should be carried out at room temperature (15–25°C).

Procedure:

1. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.

Ensure that RNase A (not provided in kit) has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet therefore mix it gently with pipetting.

2. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube several times. Do not allow the lysis reaction to proceed for more than 5 min.

Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear.

3. Add 350 µl Buffer PN3, mix immediately and thoroughly by inverting the tube several times.

To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer PN3. Large culture volumes (e.g. ≥5 ml) may require quite longer for mixing by inverting the tubes. The solution should become cloudy.

4. Centrifuge for 10 min at ≥ 10,000 rpm in a table-top microcentrifuge.

A compact white pellet will form.

5. Apply the supernatants from step 4 to the spin column by decanting or pipetting.

6. Centrifuge for 1 minute at ≥ 10,000 rpm. Discard the flow-through.

7. Recommended: Wash the spin column by adding 0.5 ml Buffer PW1 and centrifuging for 1 minute at ≥ 10,000 rpm. Discard the flow-through.

This step is necessary to remove trace nuclease activity when using endA+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have

high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5a do not require this additional wash step.

8. Wash spin column by adding 0.7 ml Buffer PW2 and centrifuging for 1 minute at ≥ 10,000 rpm.

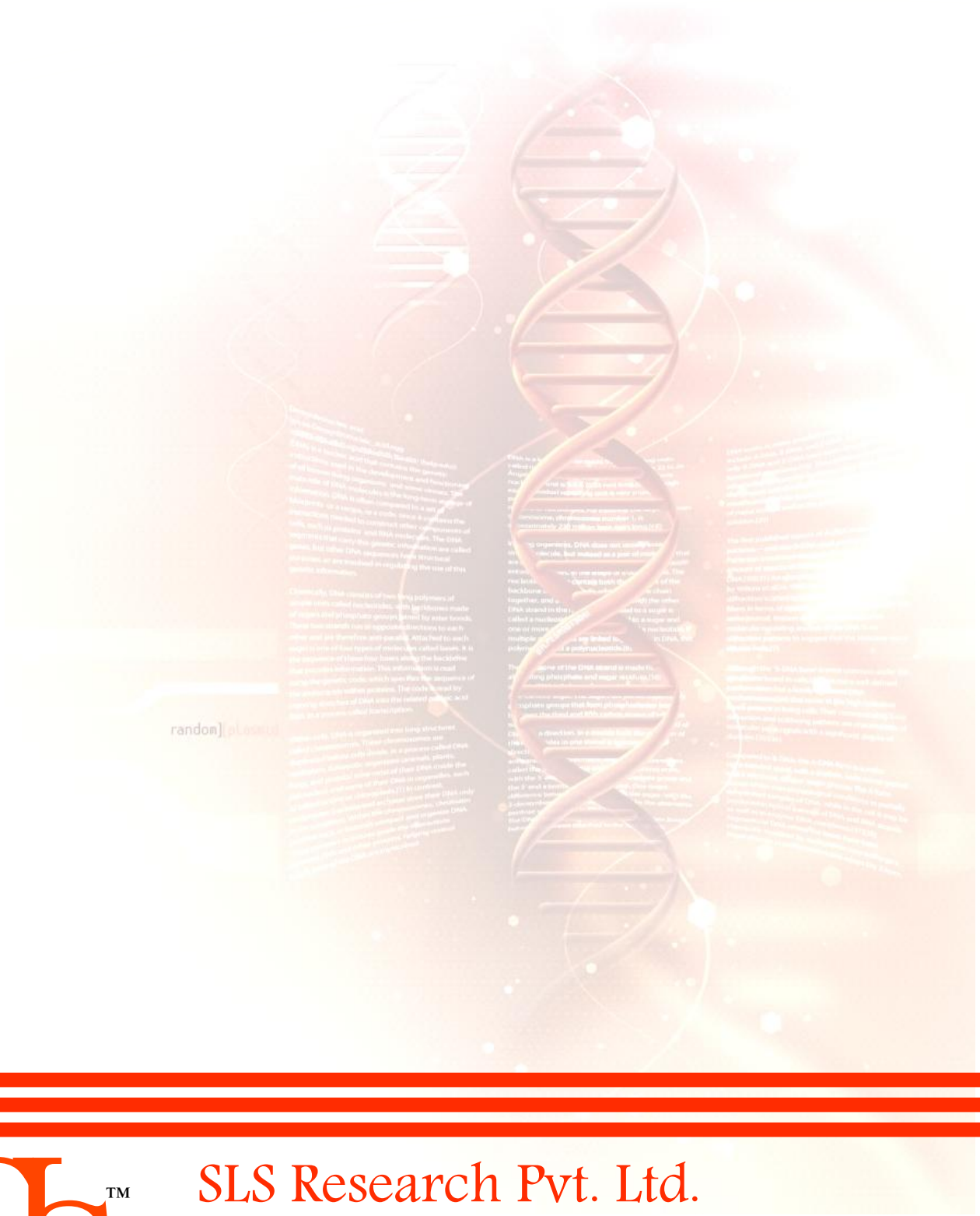
9. Discard the flow-through, and centrifuge at at ≥ 10,000 rpm for an additional 1 min to remove residual wash buffer.

Important: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PW2 may inhibit subsequent enzymatic reactions.

10. Place the column in a clean 1.5 ml microcentrifuge tube(not provided). To elute DNA, add 50 µl Buffer PE or water to the center of spin column, let stand for 1 min, and centrifuge for 1 min at ≥ 10,000 rpm.

References

1. Birnboim, H.C., and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7, 1513–1522.



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