GRS Plasmid Purification Kit - mini

#GK13.0100 (100 preps) | GK13s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : 1.5 ml of cultured bacterial cells (up to 6 ml)
Expected Yield : up to 50 µg of plasmid DNA
Format : spin column
Operation Time : within 20 minutes
Elution Volume : 30-10µl

Product: The GRS Plasmid Purification Kit provides an efficient and fast method for the purification of high-quality plasmid DNA from 1-6 ml of cultured bacterial cells. Eluted DNA is suitable for all common downstream applications including PCR, enzymatic restriction digestion, cloning and DNA sequencing.

The GRS Plasmid Purification Kit is based on a modified Alkaline Lysis method used to obtain minimal genomic DNA contaminants[1]. RNase A treatment reduces contamination with RNA. Blue Lysis Buffer is added to ensure efficient cell lysis and SDS precipitation. The buffer system is optimized to allow binding of plasmid DNA in the presence of chaotropic salts to the glass fiber matrix of the spin column[2]. Contaminants are removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified plasmid DNA is subsequently eluted by a low salt Elution Buffer or TE or water. The entire procedure can be completed in within 20 minutes without phenol/chloroform extraction or alcohol precipitation, with a typical DNA yield of 20-30µg for high-copy number plasmids or 3-10µg for low-copy number plasmids from 4,5 ml of cultured bacterial cells

QC: The quality of the GRS Plasmid Purification Kit is tested on a lot-to-lot basis by isolating plasmid DNA from a 4.5-ml overnight culture of *Escherichia coli* DH5α (OD600nm >2), harbouring pBluescript. Yields are typically over 20 µg of plasmid DNA with an A260/A280 ratio between 1.8 and 2.0. Subsequently, 1µg of the purified plasmid is digested with *EcoRI*, and fragments are analyzed by agarose gel electrophoresis.

Caution: Buffer P3 and Wash Buffer 1 contains guanidine hydrochloride which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References:

1. Birnboim, HC., and Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513-1523
2. Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615-619

Kit Contents	(100 preps)	(4 preps)
Buffer P1**	25 ml	1 ml
Buffer P2	25 ml	1 ml
Buffer P3	45 ml	1.5 ml
Blue Lysis Buffer***	250 µl	10 µl
Wash Buffer 1	45 ml	2 ml
Wash Buffer 2*	25 ml	1 ml
Elution Buffer	6 ml	1 ml
Plasmid DNA mini spin Column	100	4
1.5-ml microtube (DNase/RNase free)	200	-
2-ml collection tube	100	4
RNase A (50mg/ml)	100 µl	added

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips
Vortex
Water bath or Thermoblock

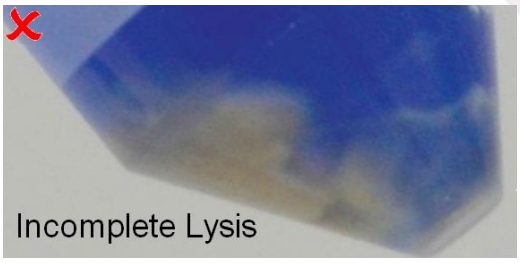
*Add Ethanol (96%-100%) [not included] to Wash Buffer 2, as indicated on the bottle, prior to initial use. After Ethanol has been added, mark the bottle to indicate that this step has been completed. Close bottle tightly to avoid ethanol evaporation.

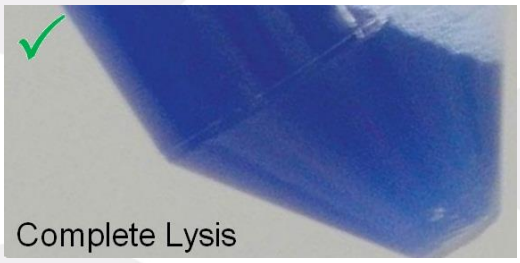
** Prior to use, add the provided RNase A solution to Buffer P1 and store Buffer P1 +4°C. In the trial sample, RNase A has already been added to Buffer P1

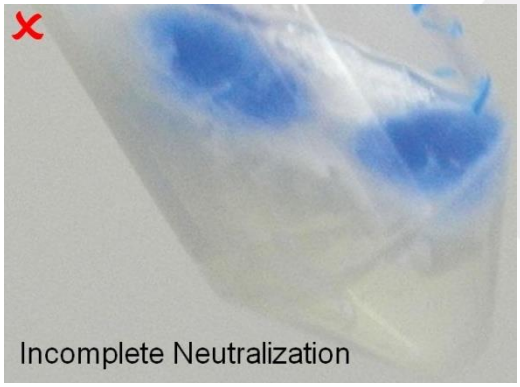
*** If desired to use Blue Lysis Buffer, add the content of the vial to Buffer P1 prior to use first time. Note that the vial containing Blue Lysis Buffer might seem empty due to solvent (ethanol) evaporation. In that case, rinse the tube with Buffer P1 in order to be able to transfer the whole content.

Storage: Upon arrival of the kit, store RNase A at -20°C. After preparation of Buffer P1 with RNase A, store Buffer P1 at +4°C. All other components should be stored at room temperature. Examine solutions for precipitates before use. Any precipitate (e.g., in Buffer P2) may be re-dissolved by warming the solution to 37°C followed by cooling to 25°C.

Blue Lysis Buffer (optional):
(for preparation of P1 + Blue Lysis Buffer, see above)







PROTOCOL FOR PLASMID DNA PURIFICATION

This protocol can be carried out with or without Blue Lysis Buffer added to Buffer P1 (see notes on page 2).

1) Transfer 1.5 ml of a culture of bacterial cells harbouring the plasmid of interest to a 1.5-ml microcentrifuge tube and harvest the cells by centrifugation at 14,000-16,000g for 1 minute. Discard the supernatant. If desired, more than 1.5 ml of cultured cells (up to 6 ml) can be used by repeating the harvesting step.

2) Add 200µl of Buffer P1 (Ensure that RNase A was added) to the bacterial pellet, and resuspend the cells by vortexing or pipetting up and down.

3) Add 200µl of Buffer P2 (check for precipitates) and mix immediately by gently inverting the tube 10 times. DO NOT VORTEX! (vortexing might lead to shearing of genomic DNA). Incubate at room temperature until lysis is complete.
Note that with Blue Lysis Buffer, after adding buffer P2, precipitates will completely dissolve and the suspension will become blue. Continue mixing in case the suspension contains brownish cell clumps or colourless regions (see pictures on page 2)

4) Neutralize the solution by adding 300µl of Buffer P3. Mix immediately by gently inverting the tube 10 times. DO NOT VORTEX! *Note that with Blue Lysis Buffer, after adding buffer P3, the suspension becomes colourless. Continue mixing in case the suspension contains blue regions (see pictures on page 2).*

5) Centrifuge at 14,000-16.000g for 3 minutes.

6) Place a Plasmid DNA Mini Spin Column into a 2-ml collection tube. Add the supernatant from step 5 to the column. Centrifuge at 14,000-16,000g for 30 seconds.

7) Discard the flow-through, and place the spin column back in the collection tube.
Add 400µl of Wash Buffer 1 and centrifuge at 14,000-16,000g for 30 seconds.

8) Discard the flow-through, and place the spin column back in the collection tube.
Add 600µl of Wash Buffer 2 (Ensure ethanol was added to the Wash Buffer 1st time prior to use)

9) Centrifuge at 14-16.000g for 30 seconds and discard the flow-through

10) Place the spin column back in the collection tube and centrifuge at 14,000-16,000g for another 3 minutes to dry the matrix of the column.

11) Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipet 50µl Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 2 minutes.
Notes: 1) Yield could be increased using pre-warmed Elution Buffer (60ºC). 2) Instead of Elution Buffer, DNA can also be eluted with TE or water; pH ideally should be 8.0-8.5.

12) Centrifuge for 2 minutes at 14,000-16,000g to elute purified plasmid DNA. Discard the spin column and use DNA immediately or store at -20ºC.

TROUBLESHOOTING

1. **Low Yield**
    - *Incomplete Lysis of Bacterial cells*
        - **i.** Too many bacterial cells. If OD_{600nm} > 10: dilute cells into multiple tubes
        - **ii.** Invert tubes more often to ensure the sample is homologous
    - *Incorrect DNA Elution Step*
        - **i.** Ensure that the Elution Buffer is completely adsorbed after being added to the center of the spin column
    - *Incomplete DNA Elution*
        - **i.** For large plasmids (>10kb), using preheated (60°C-70°C) elution buffer may improve the elution efficiency

2. **Low Quality**
    - *Low performance in downstream applications*
        - **i.** Residual ethanol contamination interferes with downstream applications. Following the wash step, dry the spin column with additional centrifugation for 5 minutes or incubation at 60°C for 5 minutes in order to evaporate ethanol.
        - ii. In case of genomic DNA contamination (which can be detected by gel analysis), make sure that mixing steps are done gently to prevent smearing. Also do not use overgrown bacterial cultures.
        - iii. RNA contamination might be due to incorrect preparation or storage of Buffer P1 (containing RNase A)