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GRS Plasmid Purification Kit
#GK13.0100

(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 :	30-100 µl

Product Description

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.

Principle

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 yields of 20-30µg for high-copy number plasmids or 3-10µg for low-copy number plasmids from 4,5 ml of cultured bacterial cells.

Quality Control

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 *Eco*RI, 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)

Buffer P1*	25 ml
Buffer P2	25 ml
Buffer P3	45 ml
Blue Lysis Buffer***	250 µl
Wash Buffer 1	45 ml
Wash Buffer 2**	25 ml
Elution Buffer	6 ml
Plasmid DNA mini spin column	100
1,5-ml microtube (DNAse/RNAse free)	200
2,0-ml collection tube	100
RNAse A (50mg/ml)	100 µl

Required Components (not included)

Ethanol (96%-100%)
Centrifuge for microtubes
Pipets (and tips)
Vortex
Waterbath or Thermoblock

Notes

*Add the provided RNAse A solution to Buffer P1 and store at +4ºC

** Add 100 ml ethanol (96%-100%) [not included] to Wash Buffer 2 prior to initial use. After ethanol has been added, mark the bottle to indicate that this step has been completed.

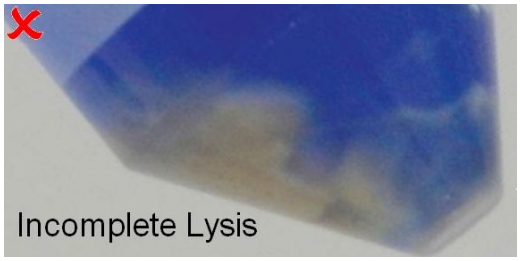
******* If desired to use Blue Lysis Buffer, add the content of the vial to Buffer P1 prior to use first time. Note that the vial containing Blue Lysis Buffer might seem empty due to solvent (ethanol) evaporation. In that case, rinse the tube with 250µl of 100% Ethanol and/or Buffer P1 in order to be able to transfer the whole content.

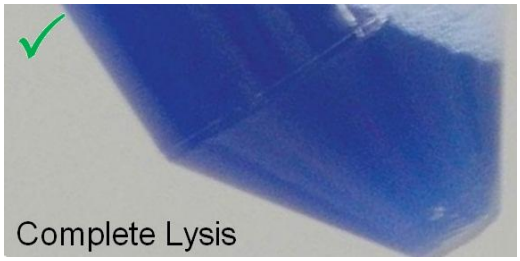
Storage

Upon arrival of the kit, store RNAse A at -20ºC. After preparation of Buffer P1 with RNAse A, store Buffer P1 at +4ºC. 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 37ºC followed by cooling to 25ºC.

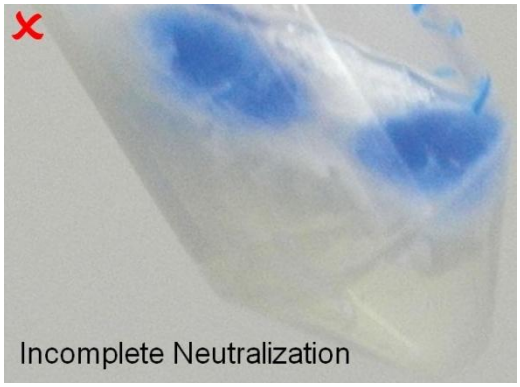
BLUE LYSIS BUFFER(OPTIONAL)
(for preparation of P1 + Blue Lysis Buffer, see above)

LYSIS:





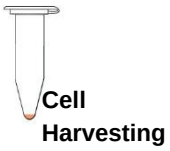
NEUTRALIZATION:



PROTOCOL FOR PLASMID DNA PURIFICATION

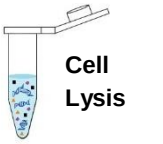
This protocol can be carried out with or without Blue Lysis Buffer added to Buffer P1 (see notes on page 2).

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 14-16.000g 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 (**check if 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 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 after adding buffer P3, the suspension becomes colourless. Continue mixing in case the suspension contains blue regions (see pictures on page 2).* Centrifuge at 14-16.000g for 3 minutes.

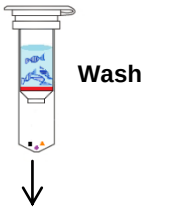
5) Place a **Plasmid DNA Mini Spin Column** into a 2,0-ml collection tube. Add the supernatant from step 5 to the column. Centrifuge at 14-16.000g for 30 seconds.

6) Discard the flow-through, and place the spin column back in the collection tube. Add **400 µl of Wash Buffer 1** and centrifuge at 14-16.000g for 30 seconds.

7) Discard the flow-through, and place the spin column back in the collection tube. Add **600µl of Wash Buffer 2*** (check if ethanol is added)

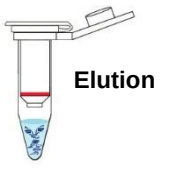
8) Centrifuge at 14-16.000g for 30 seconds and discard the flow-through

9) Place the spin column back in the collection tube and centrifuge at 14-16.000g for another 3 minutes to dry the matrix of the column.



10) Transfer the spin column to a new 1,5-ml microcentrifuge tube and pipet 50µl **Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 2 minutes.
Notes: Yield could be increased using pre-warmed Elution Buffer (60ºC-70ºC). Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5.

11) Centrifuge for 2 minutes at 14-16.000g to elute purified 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 OD600nm >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 centre of the spin column
   - *Incomplete DNA Elution*
     - i. For large plasmids (>10kb), using preheated (60ºC-70ºC) 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 60ºC 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 storage of Buffer P1.

ORDERING INFORMATION – GRS Nucleic Acid Purification Kits

Reference #	Product Name	Quantity (kit)
GK01.0100	GRS PCR & Gel Band Purification Kit	100 preps
GK02.0100	GRS Genomic DNA Kit - Blood & Cultured Cells	100 preps
GK03.0100	GRS Genomic DNA Kit – Tissue	100 preps
GK04.0100	GRS Genomic DNA Kit – Plant	100 preps
GK05.0100	GRS Pure DNA Kit	100 preps
GK06.0100	GRS Genomic DNA Kit – BroadRange	100 preps
GK07.0100	GRS Genomic DNA Kit – Bacteria	100 preps
GK08.0100	GRS Total RNA Kit - Blood & Cultured Cells	100 preps
GK09.0100	GRS Total RNA Kit – Tissue	100 preps
GK10.0100	GRS Total RNA Kit – Plant	100 preps
GK11.0050	GRS microRNA Kit	50 preps
GK12.0050	GRS Viral DNA/RNA Purification Kit	50 preps
GK13.0100	GRS Plasmid Purification Kit	100 preps

Note: Individual components (buffers, columns, tubes, enzymes) can be purchased separately. For more information, please contact us via info@grisp.pt

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