

## GRS Plasmid Purification Kit - mini

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



<b>Sample :</b>	1.5 ml of cultured bacterial cells (up to 6 ml)
<b>Expected Yield :</b>	up to 50 µg of plasmid DNA
<b>Format :</b>	spin column
<b>Operation Time :</b>	within 20 minutes
<b>Elution Volume :</b>	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<sup>1</sup>. 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<sup>2</sup>. 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 (OD<sub>600nm</sub> >2), harbouring pBluescript. Yields are typically over 20 µg of plasmid DNA with an A<sub>260</sub>/A<sub>280</sub> ratio between 1.8 and 2.0. Subsequently, 1 µg 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



## 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 1<sup>st</sup> 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 (60°C). 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 -20°C.

## 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)