

## VACUUM MANIFOLD PROTOCOL INSERT

### Very Important!

Before starting the procedure, make sure that solution G4 is reconstituted as indicated on the bottle's label.

Solution G1 contains RNase and should be stored at 4 °C.

If a precipitate has formed in solution G2, re-dissolve the precipitate (SDS) by incubating the solution in a water bath (37-45 °C) for a few minutes and gentle swirling from time to time.

All centrifugation steps are carried out at top speed (approx. 13,000 rpm) in a conventional table-top microfuge.

Do not overload the spin column. The use of 1-5 ml *E. coli* culture is recommended. The maximum amount of culture used should be 5 ml. With even higher culture volumes DNA yield and quality may decrease rapidly.

## PROTOCOL

- 1.) **Harvesting bacterial cells:** *E. coli* cells are pelleted by centrifugation. Remove all traces of medium carefully. Make sure, that culture medium back-draining from the tube's wall is removed.
- 2.) **Cell Resuspending:** Add **250 µl** of **solution G1** (Cell Suspension Buffer) to the pellet and resuspend the cells (by vortexing or with a pipette) until the suspension is homogeneous.
- 3.) **Cell Lysis:** Add **250 µl** of **solution G2** (Cell Lysis Buffer) and mix gently, but thoroughly by inverting the tube several times. Do not vortex! Incubate at room temperature for 5 min.
- 4.) **Neutralization:** Add **350 µl** of **Neutralization Buffer G3** and mix gently but thoroughly by inverting the tube until a homogeneous phase is obtained. Do not vortex! Centrifuge the mixture at room temperature and at top speed for 10 min.
- 5.) **Vacuum Manifold Preparation:** Attach the vacuum manifold to a vacuum source. Attach a JETQUICK Plasmid Miniprep cartridge to a female luer extension on the vacuum manifold. Load the clear supernatant from step 4 into the cartridge. Apply vacuum (-200 to -650 mbar) until all liquid has been pulled through the cartridge. Then turn off the vacuum source.
- 6.) **(Optional Wash):** This additional column wash is only recommended, if *E. coli* cells rich in nucleases, carbohydrates or secondary metabolites (i.e. *endA*<sup>+</sup> strains) have been used:  
  
Add **500 µl** of the **Optional Wash Buffer GX** to the cartridge and incubate at room temperature for 1 min. Apply vacuum (-600 to -650 mbar) until all liquid has been pulled through the cartridge. Then turn off the vacuum source.

- 7.) **Cartridge Wash:** Add **700 µl** of **Wash Buffer G4** (reconstituted with ethanol) to the cartridge. Apply vacuum (-600 to -650 mbar) until all liquid has been pulled through the cartridge. Then turn off the vacuum source. Place the micro-spin cartridge into a 2 ml wash tube and centrifuge at top speed for 1 min to remove residual wash buffer.
- 8.) **Plasmid Elution:** Place the JETQUICK micro-spin cartridge into a new 1.5 ml recovery tube and add **75 µl** of sterile water (or TE buffer or 10 mM Tris-HCl [pH 7-8]) directly onto the center of the silica matrix of the spin column. Centrifuge at top speed for 2 min.
- Important:** Higher DNA concentrations will be obtained if the elution is carried out with only 50 µl of elution buffer. In this case preheat the elution buffer to 65-70 °C, add the buffer onto the center of the silica matrix of the spin column and let stand for 1 min before centrifugation. Preheated elution buffer is generally recommended when plasmids larger 5 kb are eluted. DNA eluted in water should be generally stored at -20 °C.*