Bio Bharati Life Science Pvt. Ltd.

BBL Mini Plasmid Isolation Kit
Spin Column
Cat: # BB-PIK50 (50 preps)

Introduction:

BBL-Mini Plasmid Isolation Kit is a spin column based kit which is basically based on silica membrane purification technology. The silica membrane in the presence of chaotropic agent at low pH efficiently adsorbs DNA which can be easily eluted in neutral pH (~8.0).

This kit can efficiently extract and purify plasmid DNA from bacteria. The purified plasmid DNA is suitable for basic molecular biology techniques like restriction digestion, PCR amplification, sequencing etc.

Kit Contents:

Contents	# BB-PIK50 (50 preps)
Suspension Buffer	15 ml
Lysis Buffer	15 ml
Neutralization Buffer	20 ml
Wash Buffer 1	18 ml
Wash Buffer 2	15 ml
Elution Buffer	15 ml
RNase A	150 µl
Spin Column	50 nos
Collection tubes	50 nos

Storage:
BBL Mini Plasmid Isolation Kit (**# BB-PIK50)** is stable for 12 months if stored properly in dry place at room temperature (RT, 20°C - 25°C) not exposed to sunlight.

Note:
- ✓ Add RNase A in Suspension Buffer and mix well before initiating the experiment. Once mixed, store the Suspension Buffer at 4°C.

- ✓ Add ethanol (96-100%) to Wash buffer 1 and 2 before use (see bottle label for volume)

- ✓ Prolonged storage may cause visible precipitation in Lysis Buffer and Neutralization Buffer. Dissolve it at 37°C bath and cool at RT (20°C-25°C) before use.
- ✓ All centrifugation steps are carried out at 12000 rpm (~13400xg) in a conventional table top centrifuge at RT (20°C-25°C).

- ✓ Ensure that the elution buffer is dispensed directly onto the membrane for complete elution of bound DNA. The average volume of elution buffer is ~50 µl.

Protocol:

1. **In a microcentrifuge tube pellet 5 ml of overnight grown *E.coli* cells in a tabletop centrifuge for 30-60s. Remove the supernatant by pipetting. Use the same tube to spin down remaining cells and remove supernatant completely.**
   5 ml of overnight E.coli cells is recommended for high copy number plasmids. For low copy number plasmid 10 ml of overnight culture can be used and divide accordingly.

2. **Resuspend the bacterial pellet in 250 µl of Suspension Buffer.**
   Ensure that RNase A has been added as described in the "Note" section. No cell clumps should be visible after resuspension. Vortexing can help for complete suspension of the cell pellet. Presence of cell lumps will affect the lysis and lead to low yield of final plasmid DNA.

3. **Add 250 µl of Lysis Buffer and gently invert the tube 4-6 times to mix and incubate for 5 minutes**
   Do not vortex to mix as this will result in shearing of genomic DNA. If necessary invert the tubes several times until the solution becomes slightly viscous and clear. Don't allow the lysis reaction to stand more than 5 min.

4. **Add 350 µl of Neutralization Buffer and mix gently by inverting the tube 4-6 times.**
   Immediately, after addition of Neutralization Buffer the solutions become cloudy.

5. **Centrifuge for 10 min. Place a spin column in a 2 ml collection tube provided in the kit during centrifugation.**
   If supernatant is not clear enough, repeat the above step once again.

6. **Apply the supernatants from Step 5 to the spin column.**

7. **Centrifuge for 1 min and discard the flow through.**

8. **Wash the spin column with 500 µl of Wash Buffer 1 for 1 min and discard the flow through.**

9. **Wash the spin column with 700 µl of Wash Buffer 2 for 1 min and discard the flow through.**

10. Repeat the above step once again
Wash the column twice with Wash Buffer 2 to rinse out excess salt.

11. Discard the flow through and centrifuge for an additional 2 min to remove residual wash buffer

Residual wash buffer should be completely removed. As residual ethanol from Wash buffer 2 may inhibit subsequent enzymatic reaction

12. Place the spin column in the 1.5ml micro centrifuge tube. To elute the DNA add 50µl of Elution Buffer to the centre of the silica membrane. Incubate the column for 2 min at room temperature followed by centrifugation for 2 min.