High Purity Plasmid MiniPrep Kit

(100) Cat. # KTS1015

Contents:	**100 Preps**
RNase A (10 mg/ml)	(300 µl)
Resuspension Solution **(A1)**	(30 ml)
Lysis Solution **(A2)**	(30 ml)
Neutralization Solution **(A3)**	(40 ml)
Wash Buffer **B**	(50 ml)
Wash Buffer **W**	(30 ml x2)
Elution Buffer **EB**	
(10 mM Tris-HCl, pH 8.5)	(10 ml)
Spin Columns	(100 each)

- **Add provided RNase A solution to the Resuspension solution (A1) and mix. (After the addition of RNase A, Solution A1 should be stored at 4°C when not being used.)**
- **Prior to use, add 45 ml of ethanol (95-100% not provided) to each Wash Buffer W.**

Protocol

1. Pellet 1-5 ml of an overnight culture. Thoroughly remove all medium from the cell pellet.

2. Resuspend the pelleted cells in **250µl of A1**. Transfer the cell suspension to a 1.5ml microcentrifuge tube. Resuspend by votexing or pipetting up and down, until no clumps remain.
Note. Ensure RNase A has been added to A1.

3. **Add 250 µl of A2** and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear.
Note. *Do not vortex, to avoid shearing the chromosomal DNA. Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.*

4. **Add 350 µl of A3** and mix immediately and thoroughly by inverting the tube 4-6 times.
Note. *It is important to mix thoroughly and gently after the addition of Solution **A3** to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate is cloudy and viscous.*

5. **Centrifuge for 10 min at 12,000 rpm** to pellet cell debris and chromosomal DNA.

6. Transfer the supernatant to the supplied spin column. Avoid disturbing or transferring the white precipitate.

7. Centrifuge for 1 min at 12,000 rpm. Discard flow-through and place the column back into the same collection tube.
Note. *Do not add bleach to the flow-through.*

8. **Add 500 µl of the Wash Buffer B** to the spin column. Centrifuge for 30-60 seconds at 12,000 rpm and discard flow-through. Place the column back into the same collection tube.

9. **Add 750 µl of the Wash Buffer W** to the spin column. Centrifuge for 30-60 seconds at 12,000 rpm and discard flow-through. Place column back into the same collection tube.
Note. Wash Buffer W must previously be diluted with 45 ml of ethanol (95-100%).

10. Discard flow-through and centrifuge for an additional 1 min to remove residual Wash Buffer W. This step is essential to avoid residual ethanol in plasmid preps.

11. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and **pipette 50 µl (30-100µl) of Elution Buffer EB** directly to the center of the column without touching the membrane. Incubate for 1 min at room temperature and centrifuge for 1 min at 12, 000 rpm.
Note. *For elution of large plasmids or cosmids, warm Elution Buffer to 60°C before applying to silica membrane.*

12. Discard the column and store the purified plasmid DNA at -20°C.