MINIPREP KIT

- **Reagents:**
  - Miniprep kit
  - LB medium
  - Agar medium LB
  - eppendorf tubes 1.5 mL
- **Material:**
  - Micropipettes 1000 uL, 100 uL, 10 uL
  - Pipette tips 1000 uL, 100 uL, 10 uL

- **Equipment:**
  - Centrifugation

Production of a Cleared Lysate Note:

1. Harvest 1–5ml (high-copy-number plasmid) or 10ml (low-copy-number plasmid) of bacterial culture by centrifugation for 5 minutes at 10,000 x g in a tabletop centrifuge. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.
2. Add 250µl of Cell Resuspension Solution and completely resuspend the cell pellet by vortexing or pipetting. It is essential to thoroughly resuspend the cells. If they are not already in a microcentrifuge tube, transfer the resuspended cells to a sterile 1.5ml microcentrifuge tube(s). Note: To prevent shearing of chromosomal DNA, do not vortex after Step 2. Mix only by inverting the tubes.
3. Add 250µl of Cell Lysis Solution and mix by inverting the tube 4 times (do not vortex). Incubate until the cell suspension clears (approximately 1–5 minutes). Note: It is important to observe partial clearing of the lysate before proceeding to addition of the Alkaline Protease Solution (Step 4); however, do not incubate for longer than 5 minutes.
4. Add 10µl of Alkaline Protease Solution and mix by inverting the tube 4 times. Incubate for 5 minutes at room temperature. Alkaline protease inactivates endonucleases and other proteins released during the lysis of the bacterial cells that can adversely aff ect the quality of the isolated DNA. Do not exceed 5 minutes of incubation with Alkaline Protease Solution at Step 4, as nicking of the plasmid DNA may occur.
5. Add 350µl of Neutralization Solution and immediately mix by inverting the tube 4 times (do not vortex).
6. Centrifuge the bacterial lysate at maximum speed (around 14,000 × g) in a microcentrifuge for 10 minutes at room temperature.

Centrifugation protocol:

- Production of Cleared Lysate

1. Isolation by cross streaking on petri dish until getting completely separated colonies.
2. Inoculate a single colony into a flask containing 50 mL of LB medium. Incubate overnight at 37 ° C in a rotary shaker (250 rpm).
3. Pellet 1–10 ml of overnight culture for 5 minutes.
4. Thoroughly resuspend pellet with 250µl of Cell Resuspension Solution
5. Add 250µl of Cell Lysis Solution to each sample; invert 4 times to mix
6. Add 10µl of Alkaline Protease Solution; invert 4 times to mix. Incubate 5 minutes at room temperature.
7. Add 350µl of Neutralization Solution; invert 4 times to mix.
8. Centrifuge at top speed for 10 minutes at room temperature.

- Binding of Plasmid DNA

9. Insert Spin Column into Collection Tube.
10. Decant cleared lysate into Spin Column
11. Centrifuge at top speed for 1 minute at room temperature. Discard flowthrough, and reinsert Column into Collection Tube.

- Washing

12. Add 750µl of Wash Solution (ethanol added). Centrifuge at top speed for 1 minute. Discard flowthrough and reinsert column into Collection Tube.
13. Repeat Step 10 with 250µl of Wash Solution.
14. Centrifuge at top speed for 2 minutes at room temperature.

- Elution

15. Transfer Spin Column to a sterile 1.5ml microcentrifuge tube, being careful not to transfer any of the Column Wash Solution with the Spin Column. If the Spin Column has Column Wash Solution associated with it, centrifuge again for 1 minute at top speed, then transfer the Spin Column to a new, sterile 1.5ml microcentrifuge tube.
16. Add 80µl of Nuclease-Free Water to the Spin Column. Centrifuge at top speed for 1 minute at room temperature.
17. Discard column, and store DNA at –20°C or below.