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May 21, 2020

Plasmid DNA Mini Kit I- Spin Protocol

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1 *Works for me* This protocol may be deleted by the owner

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1 1) Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an endA negative strain of *E. coli*be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®.

2 2) Centrifuge at 10,000 x g for 1 minute at room temperature.

3 3) Decant or aspirate and discard the culture media.

4 4) Add 250 µL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.
Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section.

5 5) Transfer suspension into a new 1.5 mL microcentrifuge tube.

6 6) Add 250 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO2 in the air.

7 7) Add 350 µL Solution III. Immediately invert several times until a flocculent white precipitate forms. **Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.**

8 8) Centrifuge at maximum speed (≥13,000 x g) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

9 9) Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

(BREAK)

10 ***Preparing Column Prior to use (non-optional)***

Protocol for Column Equilibration:

1) Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.

11 2) Centrifuge at maximum speed for 30-60 seconds.

12 3) Discard the filtrate and reuse the collection tube.

13 4) Rinse with 200 uL of Neutralization buffer (P3)

14 ***(CONTINUATION of Step 9)***

10) Transfer the cleared supernatant from Step 8 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

15 11) Centrifuge at maximum speed for 1 minute.

16 12) Discard the filtrate and reuse the collection tube.

17 13) Add 500 µL HBC Buffer.

18 14) Centrifuge at maximum speed for 1 minute.

19 15) Discard the filtrate and reuse collection tube.

20 16) Add 700 µL DNA Wash Buffer.

Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use

21 17) Centrifuge at maximum speed for 1 minute.

22 18) Discard the filtrate and reuse the collection tube.

Optional: Repeat Steps 16-18 for a second DNA Wash Buffer wash step.

23 19) Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

Note: It is important to dry the HiBind® DNA Mini Column matrix before elution.*Residual ethanol may interfere with downstream applications.*

24 20) Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

25 21) Add 30-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

26 22) Let sit at room temperature for 1 minute.

27 23) Centrifuge at maximum speed for 1 minute.
Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

28 24) Store DNA at -20°C.