



Plasmid DNA extraction V.3

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¹2021 iDEC NEFU_China

Version 3 ▼

1 Works for me

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ABSTRACT

This protocol is used to extract plasmid DNA from E. coli.

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GUIDELINES

This protocol is based on E.Z.N.A.® Plasmid DNA Mini Kit I Protocol - Spin Protocol.

MATERIALS TEXT

- 100% ethanol
- Isopropanol
- Microcentrifuge capable of at least 13,000 x g
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Culture tubes
- Optional: sterile deionized water
- Optional: water bath or incubator capable of 70°C
- Optional: 3M NaOH solution


SAFETY WARNINGS

Please wear gloves before start.

BEFORE STARTING

Prepare DNA Wash Buffer, HBC Buffer, and Solution I .

1. Add the vial of RNase A to the bottle of Solution I and store at 2-8°C;
2. Dilute DNA Wash Buffer with 100% ethanol 120ml and store at room temperature;
3. Dilute HBC Buffer with isopropanol and store at room temperature;
4. Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37°C.

- 1 Solate 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 Centrifuge at 10,000 x g for 1 minute at room temperature. 1m
 **10000 x g, Room temperature , 00:01:00**
- 3 Decant or aspirate and discard the culture media.
- 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.

RNase A must be added to Solution I before use.

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

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

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 Add 350 μ L Solution III. Immediately invert several times until a flocculent white precipitate forms.

It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.

- 8 Centrifuge at maximum speed ($\geq 13,000 \times g$) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

10m

 **15000 x g, Room temperature , 00:10:00**

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

- 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.

- 11 Centrifuge at maximum speed for 1 minute.

1m

 **15000 x g, Room temperature , 00:01:00**

- 12 Discard the filtrate and reuse the collection tube.

- 13 Add 500 μ L HBC Buffer

HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

14 Centrifuge at maximum speed for 1 minute.
🌀 **15000 x g, Room temperature , 00:01:00**

15 Discard the filtrate and reuse collection tube.

16 Add 700 μ L DNA Wash Buffer.

17 Centrifuge at maximum speed for 1 minute.
🌀 **15000 x g, Room temperature , 00:01:00**

1m

18 Discard the filtrate and reuse the collection tube.

19 Repeat step 16~18 once.

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

21 Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

22 Add 30-100 μ L Elution Buffer or sterile deionized water directly to the center of the column membrane.

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.

23 Let sit at room temperature for 1 minute.

24 Centrifuge at maximum speed for 1 minute.

1m

 **15000 x g, Room temperature , 00:01:00**

This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

25 Suck out the solution from the tube and re-add it to the center of the column membrane to give a second centrifuge.

1m

 **15000 x g, Room temperature , 00:01:00**

26 Test the concentration and purity of DNA using NanoDrop.

27 Store DNA at -20°C.