Plasmid Extraction Mini Kit (Magnetic bead method)

Cat: DM1100

Size: 50T/100T

Storage: 2-8℃**(Note: RNase A will be shipped as an accessory, please store at -20℃; Magnetic bead, stored at 4℃, do not freeze)**

Kit Components:

Kit Components	50T	100T	Storage
RNase A	100μL	100μL×2	-20℃
Solution I	6mL	12mL	RT
Solution II	12mL	24mL	RT
Solution III	8mL	16mL	RT
Washing Buffer	20mL	20mL×2	RT
Elution Buffer	15mL	30mL	RT
Magnetic bead	750μL	1.5mL	2-8℃, do not freeze

Note：Please add absolute ethanol to the washing buffer before use. Please refer to the label on the bottle for the added volume (Each bottle needs to add 60mL absolute ethanol).

Introduction:

The kit uses the magnetic bead method to extract plasmid DNA. The special chemical groups on the surface of the magnetic bead can be specifically adsorbed with DNA, and high-quality plasmid DNA can be extracted, and the operation is simple. The genomic DNA extracted with this kit can be applied to a variety of routine molecular biology experiments, including enzyme digestion, PCR, sequencing, linking and transformation tests. This kit is safe to operate without the use of toxic reagents.

Protocols (*only for reference*):

1. Take 1-5mL bacterial cultures, centrifuge for 1min at 12000rpm, remove supernatant (if there are too much bacterial liquid, bacteria can be collected into a centrifuge tube by multiple centrifugation).
2. Add 100uL Solution I and 2μL RNase A to centrifuge tube. Suspend the bacterial cell precipitate thoroughly by pipette or vortex oscillator (If it is not thoroughly mixed, it will affect the lysis and lead to the low amount and purity of the plasmid extraction), put at room temperature for 5-10min. The corresponding volume of RNA enzyme can be added to the solution according to the requirement of solution I. (Recommend it is to make when it is in need).
3. Add 200uL Solution II to centrifuge tube. Invert and gently rotate the tube 6-8 times to fully lyse the bacteria.
4. Add 150uL Solution III to centrifuge tube, immediately and gently invert 6-8 times to mix well, and white flocculent precipitate will appear. Centrifuge at 12000rpm for 10min, transfer the

supernatant into a clean tube, please avoid to absorb precipitate.

5. Add equal volume of anhydrous ethanol and 15μL magnetic bead to the supernatant obtained in the previous step **(the magnetic beads need to be mixed before use)**. After mixing well, put for 10min, and mix it every 2-3min during this period (or mix it with a mixing device for 10min).

6. Put the centrifugal tube on the magnetic separation device. After the solution is clarified, absorb the supernatant. Be careful not to absorb the magnetic beads.

7. Add 600μL washing buffer to the centrifugal tube **(please confirm whether absolute ethanol has been added before use)**, swirl and mix well, immediately put on the magnetic separation device for adsorption, and then suck out the liquid after the magnetic beads collect liquid and clarify.

8. Repeat step 7.

9. Place the centrifuge tube on the magnetic separation device with the cover open for 5min to remove excess ethanol. If there is excess liquid at the bottom of the centrifuge tube during the standing period, use the pipette to suck it out.

10. Add 50-200uL elution buffer which is after 65℃ water bath to the centrifuge tube, mix well with a pipette gun, put at room temperature for 5min, and then place on the magnetic separation device for adsorption. After the liquid collected by the magnetic beads was clarified, the liquid was sucked into a clean centrifuge tube, and the liquid was the extracted plasmid DNA.

Notes：

1. In the process of extracting plasmid, if you want to increase the concentration of plasmid, the amount of bacteria can be appropriately increased.

2. Check Solution II, Solution III before use. If the buffer appears turbid, dissolve it by warming at 37℃ for several minutes, they can be used until become clear. Keep the cover of Solution II, Solution III tightly after use.

3. If the volume of elution buffer is less than 50uL, it may affect recovery efficiency. The pH value of elution buffer has influence in eluting too. If using water as elution buffer, pH should be controlled at 8.0 (adjusted by NaOH), pH below 7.0 will affect elution efficiency. The extracted DNA should be at -20℃ to prevent degradation.

4. DNA concentration and purity detection: The extracted DNA fragments can be detected for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. DNA should have a significant absorption peak in OD260, OD260=1 is equal to 50ug/mL double-stranded DNA, 40ug/mL single-stranded DNA. OD260/OD280 should be 1.7-1.9, the value will be lower if using distilled water in eluting, because the pH and ions will affect the light absorption value, but do not show the purity is low.