YORBIO Plasmid DNA Purification Kit

The Kit is designed for purification of plasmid DNA from 1-2 ml (high copy number), or up to 10 ml (low copy number) of *E.coli* overnight culture.

Components	D0010	D0011	D0012
Spin Columns	3	50	250
2-ml Collection Tubes	3	50	250
Rnase A, 5 mg/ml	20 µl	300 µl	1.5 ml
Solution I, resuspension buffer	0.5 ml	12 ml	60 ml
Solution II, lysis solution	0.5 ml	12 ml	60 ml
Solution III, neutralisation solution	1 ml	24 ml	2x60 ml
Wash Solution, concentrate	1.05 ml	17.5 ml	5x17.5 ml
Elution Buffer, 5 mM Tris-Cl, pH 8.0	0.2 ml	5 ml	25 ml

Notes before use:

1. Add RNase A to Solution I. Then Solution I should be stored at +4 °C.
2. Solution II may form a precipitate. Dissolve the precipitate by warming at 37-50 °C.
3. Add 2.3 volumes of ethanol (96-100%) to 1 volume of Wash Solution Concentrate, e.g. 2.5 ml of ethanol to 1.05 ml, or 41 ml to 17.5 ml.
4. TE buffer is not recommended for elution.
5. Benchtop microcentrifuge (7,000-12,000 rpm) can be used for all following procedures.
6. Optimum volume of overnight culture is 2 ml, incubate with Solution I for up to 5 minutes when using bigger volumes of culture.

Protocol:

1. Pellet cells from overnight culture (1-2 ml) in 1.5 ml or 2 ml microfuge tube with centrifuging for 15 seconds. Remove supernatant completely.
2. Add 150 µl of Solution I and vortex to resuspend the pellet.
3. Add 150 µl of Solution II, mix by gentle inverting (3-7 times) or shaking. Do not vortex! Mixture gets clear with lysis of cells. Do not incubate for longer than 1 minute.
4. Add 300 µl of Solution III and mix immediately. Incubate at room temperature for 1 minute.
5. Centrifuge for 7-10 minutes.
6. Transfer the supernatant to the column inserted into collection tube. Spin for 30 seconds.
7. Discard the flow-through liquid. Add 500 µl of Wash Solution to the column. Spin for 15 seconds. Make sure there is no liquid in the column.
8. Repeat wash step 7.
9. Discard the flow-through in the collection tube. Centrifuge for 1 minute to remove residual Wash Solution.
10. Transfer the column into 1.5 ml microfuge tube. Add 50 µl of Elution Buffer on to the center part of membrane of the column and incubate for 2 min at room temperature. Use preheated Elution Buffer (37-60 °C) for higher DNA recovery. Spin for 1 min.
11. Transfer the plasmid DNA into a clean tube and store at –20 °C.

Troubleshooting:

RNA traces in final preparation	RNase digestion was insufficient. Check culture volume against recommended volumes. Incubate with RNase for longer. Add more RNase if solution is more than 6 months old.
Low yield of plasmid DNA	a) Alkaline lysis was insufficient due to higher than recommended amount of cultured medium. Reduce culture volume or increase volumes of Solutions I, II, and III.
	b) Incubate with Elution Buffer for longer time. Add the buffer directly to the center of the column's filter. Preheat buffer and column up to 60 °C before elution step for 3-5 minutes. Centrifuge at lower speed 4,000-5,000 rpm/min.

For research use only.