

07 Extraction of Plasmid

TJUSLS China¹

¹Tianjin University



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TJUSLS China **Tianjin University**

MATERIALS

NAME

CATALOG #

VENDOR

TIANprep Mini Plasmid Kit

Collect the E. coli solution into the EP tube. Centrifuge at 12,000 rpm in a rotor for 1 minute. Remove the clear supernatant liquid.

© 00:01:00

Add 250µL P1 (RNase A added, kept at 4 °C) to the EP tube to suspend bacterial precipitation.

84°C

■250 μl

Add 250µL P2 to the EP tube, shake slightly up and down 6-8 times to lyse bacteria.

⊒250 µl

Add 350μL P3 and invert the tube immediately and gently 6-8 times. Then centrifuge it at 12000rpm, 25°C for 10 minutes.

■350 μl

8 25 °C

© 00:10:00

Regenerate column CP3 while centrifugation. Add 500µl Buffer BL. Centrifuge for 1 min at 12,000 rpm. Discard the flowthrough.

⊒500 μl

Move the clear supernatant liquid to CP3, at 12000rpm, 25°C centrifuge for 30 seconds.

8 25 °C

© 00:00:30

Add 600µL PW to adsorption column CP3, 12000rpm, 25°C centrifuge for 30 seconds.

© 00:00:30

A 25 °C

Repeat step 7.

@ 00:00:30

& 25 °C

9 Move the adsorption column CP3 to new clean centrifuge tubes and then keep them opening for 5 minutes, so that the ethanol in the PW can be sufficiently volatilized.

© 00:05:00

10 Drop 50µL 65°C ddwater into the middle of the adsorption membrane, static for 2min. Then centrifuge for 2 min at 12,000 rpm to collect DNA solution in EP tube.

⊒50 μl

8 65 °C

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